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COLOUR DIMORPHISM IN ODONATA

By

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[Received on 30th May, 1959]

R. J. Tillyard (1917) in his book "The Biology of Dragonflies" mentioned the occurrence of a very interesting phenomenon in the group of Agrioninae in which two forms of females, nearly always, occur. One form of female is commoner than the other. The commoner form is called the "normal", the rarer one the "heteromorphic". Either of these may be similar to the male (homochrome) or unlike it (heterochrome). To this phenomenon he has given the name "Colour Dimorphism" which is quite different to what is known as Sexual Dimorphism in colour (i. e. colour difference in the male and female).

The authors have also observed the occurrence of this phenomenon of colour dimorphism in two genera of the sub-family Libellulinae, but only in the males.

In *Bradinopyga geminata* (Rambur) two forms of males have been collected, one is commoner and the other rarer. The commoner form is "normal" and is similar to the female (homochrome), while the rarer form is "heteromorphic" and is unlike the female (heterochrome).

The "normal" homochrome male has a pale yellowish-white labium which often shows brownish patches. The labrum is dark pale brown with a broad yellow border which in turn again is bordered by reddish-brown. The frons and the remaining face is olivaceous. The colour of the vesicle and

occiput is brown Thorax is granite coloured *i.e.* on a dirty pale back-ground it is marbled with grey and black in a very irregular plan giving a patchy appearance. Prothorax shows two transverse black markings on the dorsal side, one at the anterior margin and the other at the posterior margin. Wings are hyaline and pterostigma is bicolourous *i.e.* black in the middle and pure white at the proximal and distal ends, between black nervures. Abdomen shows colouration similar to that of the thorax but the pattern formed by black and grey marbling on yellow base is regular. The segments three to eight of the abdomen show a definite plan. Each of these segments has a dark pale basal annule which is interrupted on dorsum by two sets of elongated black spots which run parallel to each other. One is on the ventro-lateral side and the other is sub-dorsal in position. The sub-dorsal spots again consist of two patches. One is mid-dorsal in position and dark pale in colour and the other is black triangular apical spot which is sub-dorsal in position. Anal appendages are yellowish-white in colour.

White waxy pruinescence is strongly developed on the underside of the thorax and on the ventral side of abdomen chiefly in the region of the male secondary copulatory apparatus, but extends only upto the seventh segment.

The other form or the "heteromorphic" heterochrome has a dark reddish-brown labium with two pale spots at the sides. The labrum is pale brown in the middle and pale yellowish-brown at the sides, with a pale border which again is finely bordered with reddish-brown. Frons and the rest of the face are blackish-brown. Vesicle and occiput are brown black. Eyes are dark brown. Prothorax is pale grey with black patches distributed irregularly throughout the region Thorax is dark pale greyish-black. Wings are hyaline with bicolourous pterostigma which is black in the middle and yellowish-white at the proximal and distal ends, between the black nervures. Abdomen is coloured very similarly to thorax. It is black spotted with dark pale on dorsum. The black marbling of abdomen is very regular. Segments three to eight are typical with pale grey annules whose continuity is broken by two groups of black elongated parallel spots on the dorsum. One group is ventro-lateral in position and the other is sub-dorsal. The ventro-lateral black spots are broad and elongated covering most of the area of the side. The sub-dorsal spots are black and rectangular, biggar than those found in the "normal" homochromic male. These spots occupy more than half of the dorsal area of each segment on its posterior margin. The remaining anterior half of each typical segment is dark greyish-pale with a mid-dorsal black ridge. Anal appendages are pale creamy-white in colour.

White waxy pruinescence is well developed on the under and the lateral sides of thorax and also on the ventral side of abdomen.

In *Diplacodes trivialis* (Rambur) also two forms of males occur, one commoner or "normal" and similar to the female (homochrome); while the other rarer "heteromorphic" form unlike the female (heterochrome). The colour pattern of the "normal" homochrome is as follows :

The labium is yellowish-white and the labrum is pale yellow brown. The mandible bases are creamy-yellow. Vesicle is coloured palest azure-blue. Face and frons are light azure-blue with a very fine black line at the base of the frons. Prothorax is pale greenish-yellow with a mid-dorsal yellow stripe extending full length of the dorsal side, which is flanked by two broad, elongate and parallel greyish-brown spots. Thorax is greenish-yellow or olivaceous with sutures coloured finely black. A mid-dorsal yellow stripe extends also throughout the length of the thorax. The space between mid-dorsal carina and humeral sutures is coloured

violet-brown and is spotted with minute black dots. Wings are hyaline with a yellow point in the cubital space of hind-wing. Pterostigma is unicolourous *i.e.* pale yellow between dark black nervures. Abdomen is pale greenish-yellow with all the sutures marked finely with black. In segments two and three, there are mid-dorsal and subdorsal black stripes extending from jugal suture on second segment and expanding broadly at the apical borders of segments two and three. Segments four to seven are pale yellow with elongated mid dorsal, sub-dorsal and ventro-lateral black spots expanding broadly at the apical regions of each segment. Remaining segments are black with two elongate parallel yellow spots on sub-dorsum. Anal appendages are pale or olivaceous in colour.

The "heteromorphic" heterochrome form has yellowish-white labium bordered light azure-blue. Labrum is pale yellow. Frons and face are greenish-blue with a transverse pale marking at the base of frons. Vesicle is palest azure-blue coloured. Prethorax is blue greenish-grey with a mid-dorsal yellowish-green stripe running through the whole length on the dorsum. Thorax is greenish-grey with a yellow green stripe in the mid-dorsal line similar to that found in the prothorax. Wings are hyaline with a unicolourous pterostigma which is pale brown in between dark black nervures. Cubital space of the hindwing has a small yellow point. Abdomen is dark brown black. Segments first and second are greenish-yellow with sutures marked prominently black. Remaining segments are more or less completely brown black. Anal appendages are pale yellow.

Bluish-white pruinescence is strongly developed in the whole of the thorax and also in the anterior part of abdomen both on the ventral and dorsal sides.

Summarizing, the "heteromorphic" heterochrome males are darker in colour than "normal" homochrome ones, so much so that they can easily constitute separate species taking only the colouration of the body in consideration.

DISCUSSION

It is likely that the difference in colour of the two forms of males may be attributed to the development of pruinescence, but this is not the case, as is evidenced by the following :

1—According to Imms (1951) "Pruinescence is easily removed by rubbing and wear." In the species mentioned above the colour does not change even on thorough rubbing.

2—Pruinescence is never present in the head region. The different colour of the mouth-parts, frons and face of the "heteromorphic" heterochrome males cannot, therefore, be due to the development of pruinescence.

3—In the genera discussed in the present paper, pruinescence is only present on the thorax and the anterior ventral region of abdomen. The rest of the abdomen, particularly on the dorsal side, is devoid of this exuded pigmentation. As such, the different colour of the abdomen of "heteromorphic" heterochrome males cannot be due to the presence of pruinescence.

4—Wings are never pruinosed. The different coloured pterostigma is definitely a distinct feature of the rarer form of males.

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STUDIES ON THE NUTRITION OF FUNGI

IV. THE INFLUENCE OF DIFFERENT SOURCES OF NITROGEN ON THE GROWTH OF THREE ANTHRACNOSE FUNGI

By

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[Received on 14th February, 1959]

The second paper in the series gives an account of the influence of different sources of nitrogen on the growth and sporulation of *Colletotrichum capsici* (Thind and Randhawa, 1957). The present work deals with the nitrogen nutrition and also the effect of pH on the utilization of potassium nitrite by three more anthracnose fungi, *Gloeosporium psidii*, *G. piperatum* and *Colletotrichum* sp*.

MATERIAL AND METHODS

The material and methods were the same as already described in the third paper in the series (Thind and Rawla, 1958). Unless otherwise stated the basal medium comprising dextrose 10 gm., KNO_3 5gm., KH_2PO_4 5gm., $MgSO_4$. 7 H_2O 1 gm., $Fe_2(SO_4)_3$. 6 H_2O 0·005gm. and distilled water 1,000 ml., was employed in the present investigation on the nitrogen nutrition of these pathogens.

EXPERIMENTAL WORK

Twenty-eight nitrogenous compounds, comprising 6 inorganic, 20 amino acids and 2 amides were tested as sole sources of nitrogen for the mycelial growth of these pathogens. Potassium nitrate was replaced by various nitrogenous compounds so as to provide 693 mg. of nitrogen per litre, which amount of nitrogen is present in 5 gm. of KNO_3. The basal medium (excluding KNO_3) as well as various nitrogen solutions in distilled water were sterilized at 10 lbs. pressure for 10 minutes separately and then mixed together aseptically. The initial pH of the media was adjusted to 6.

For the effect of pH on the utilization of KNO_3, the basal medium containing dextrose 20 gm., KNO_3 8·41 gm., KH_2PO_4 5 gm., $MgSO_4$. $7H_2O$ 1 gm., $Fe_2(SO_4)_3$. $6H_2O$ 0·005 gm. and distilled water 1000 ml., was employed. After autoclaving the media were adjusted to the whole range of pH with hydrochloric acid and potassium hydroxide, using a Beckman pH meter.

The media after seeding with 1 ml. of the standardized spore suspensions of these pathogens were incubated for 10 days at 28°C. These conditions have been found to be optimum for the growth of these fungi by preliminary experiments. After this period the data on dry weight of the mycelium and final pH were determined which are given in tables 1—3.

* Collected on *Citrus aurantifolia* and *C. limon* and probably is a new species (Thind and Rawla, Indian Phytopathology in Press).

Inorganic Nitrogenous Compounds :—

The data summarized in table 1 indicate that good growth of all the three pathogens occurred with almost all the inorganic nitrogenous compounds except nitrites. *Colletotrichum* sp did not grow at all on nitrites at pH 6 while *G. psidii* and *G. piperatum* yielded poor growth on nitrites at this pH. The growth was floating and greyish brown with all these pathogens with ammonium chloride; greyish black with ammonium sulphate; greyish white with ammonium nitrate only in the case of *G. psidii* and *G. piperatum* but lighter pink in the case of *Colletotrichum* sp. alone.

TABLE 1

Growth of *G. psidii*, *G. piperatum* and *Colletotrichum* sp. in media containing different inorganic nitrogenous compounds and two amides as sole sources of nitrogen, after 10 days of incubation at 28°C. Initial pH adjusted to 6.

Nitrogen Sources	*G. psidii*		*G. piperatum*		*Colletotrichum* sp.	
	Mean Dry Weight (mg.)	Final pH	Mean Dry Weight (mg.)	Final pH	Mean Dry Weight (mg.)	Final pH
Control	—	6·0	—	6·0	—	6·0
Ammonium chloride	170	2·7	180	2·7	180	3·0
Ammonium nitrate	110	5·0	120	5·6	150	6·2
Ammonium sulphate	190	2·8	190	2·8	210	2·0
Potassium nitrate	140	6·7	140	6·2	150	7·5
Potassium nitrite	42	6·0	42	6·0	—	6·0
Sodium nitrite	25	6·0	25	6·0	—	6·0
Asparagine	130	6·9	150	7·1	200	7·2
Urea	105	6·9	130	7·3	140	7·4

Effect of Hydrogen-Ion Concentration on the Utilization of KNO_2 :—

The results given in table 2 show that nitrites are utilized by these fungi only in the suitable alkaline medium, though slight growth took place with *G. psidii* and *G. piperatum* in the slightly acidic medium. All the three pathogens failed to grow at pH 3-5 and pH 11 while they yielded excellent growth at pH 8-9. Growth at pH 7 was as good as at pH 8-9 in the case of *G. psidii* and *G. piperatum*, but *Colletotrichum* sp. showed much less growth at this pH than at pH 8. Growth at pH 10 was much less than that at pH 8-9 in the case of *Colletotrichum* sp. but *G. psidii* and *G. piperatum* showed as much growth at pH 10 as at pH 8-9. At pH 7-10, the growth was floating and dull white with *G. psidii* and *G. piperatum* but with *Colletotrichum* sp. the growth was totally submerged and creamy.

TABLE 2

Effect of hydrogen-ion concentration on the utilization of KNO_3 by three anthracnose fungi. Data after 10 days incubation at 28°C.

	G psidii		*G. piperatum*		*Colletotrichum* sp.	
Initial pH	Mean Dry Weight (mg.)	Final pH	Mean Dry Weight (mg.)	Final pH	Mean Dry Weight (mg.)	Final pH
3·0	—	3·0	—	3·0	—	3·0
4·0	—	3·9	—	3·9	—	3·9
5·0	—	5·1	—	5·1	—	5·1
6·0	48	6·2	48	6·2	—	6·2
7·0	288	7·2	325	7·7	175	7·5
8·0	375	7·9	335	8·4	298	7·9
9·0	372	8·4	313	8·3	290	7·9
10·0	371	8·5	293	7·8	240	7·7
11·0	—	9·0	—	9·0	—	9·0

Organic Nitrogenous Compounds :—

As is indicated in table I the two amides (urea and asparagine) supported fairly good and greyish white growth with all these fungi.

Amino-Acids :—

The data presented in table 3 show that fair to good growth of all these pathogens occurred with almost all the amino acids except l-cystine, which gave poor growth in each case. Poor growth, however, took place with *G. psidii* only on glycine, dl-leucine, l-leucine, dl-alanine and dl-norvaline ; with *Colletotrichum* sp. on dl-norvaline, dl-lysine monohydrochloride and l-tryptophane. The Three pathogens made a dull white to creamy growth with all these amino acids, but *G. psidii* showed pale yellowish growth with l arginine monohydrochloride, orange yellow with dl-alanine, glycine, and dl-threonine and *Colletotrichum* sp. made dull brown growth with l-alanine and slightly blackish with l-glutamic acid and dl-aspartic acid. Floating mycelial mat of all these pathogens was observed with almost all the amino acids, but totally submerged growth was observed only in the case of *Colletotrichum* sp. with l-tyrosine, dl-methionine, dl-lysine monohydrochloride, l-arginine monohydrochloride and dl-norvaline.

TABLE 3

Growth of *G. psidii*, *G. piperatum* and *Colletotrichum* sp. in media containing amino acids as sole sources of nitrogen after 10 days incubation at 28°C. Initial pH adjusted to 6.

Amino Acids	*G. psidii*		*G. piperatum*		*Colletotrichum* sp.	
	Mean Dry Weight(mg.)	Final pH	Mean Dry Weight (mg.)	Final pH	Mean Dry Weight (mg.)	Final pH
Control	—	6·0	—	6·0	—	6·0
A. MONOCARBOXYLIC MONOHYDROXY ACIDS						
Glycine	80	4·6	150	7·4	140	6·8
dl-alanine	70	6·8	167	6·9	142	7·0
l-alanine	140	6·[illegible]	180	6·0	165	6·7
dl-leucine	50	5·0	170	6·8	20[illegible]	7·3
l-leucine	7[illegible]	4·0	180	6·2	160	4·2
dl-norleucine	120	5·6	160	7·4	15[illegible]	5·[illegible]
dl-valine	175	5·3	205	8·0	21[illegible]	6·3
dl-norvaline	65	6·1	14[illegible]	6·5	[illegible]	5·6
B. HYDROXY MONOAMINO ACIDS						
dl-serine	130	[illegible]	150	[illegible]·8	175	6·3
dl-threonine*	120	3·[illegible]	2[illegible]0	5·7	166	4·4
C. SULPHUR COTAINING AMINO ACIDS						
l-cystine*	50	5·2	25	6·0	30	6·1
dl-methionine	120	4·4	110	4·6	120	4·1

* These compounds were incompletely soluble.

TABLE 3—(*Contd*)

Growth of *G. psidii*, *G. piperatum* and *Colletotrichum* sp. in media containing amino acids as sole sources of nitrogen after 10 days incubation at 28°C. Initial pH adjusted to 6.

Amino Acids	*G. psidii* Mean Dry Weight (mg.)	*G. psidii* Final pH	*G. piperatum* Mean Dry Weight (mg.)	*G. piperatum* Final pH	*Colletotrichum* sp. Mean Dry Weight (mg.)	*Colletotrichum* sp. Final pH
D. MONOCARBOXYLIC DIAMINO ACIDS						
l-arginine. HCl.	120	7·8	140	8·0	160	6·8
dl-lysine. HCl.	165	4·5	120	4·5	35	5·1
E. DICARBOXYLIC MONOAMINO ACIDS						
dl-aspartic acid	120	8·2	180	8·5	160	8·5
l-glutamic acid	190	6·2	105	8·7	120	8·5
F. AROMATIC HOMOCYCLIC DERIVATIVES						
dl-b-phenylalanine	170	5·4	135	6·5	200	4·5
l-tyrosine*	130	4·2	240	6·7	140	5·1
G. AROMATIC HETEROCYCLIC DERIVATIVES						
l-tryptophane	170	7·4	250	8·0	40	6·2
l-histidine HCl.	140	6·3	170	7·5	110	4·1

* These compounds were incompletely soluble.

DISCUSSION

Nitrites are considered to be toxic to fungi in the acid medium. However, Tandon and Aggarwal, 1953, Morton and MacMillan, 1954, Thind and Sandhu, 1956, have observed the utilization of nitrites by *Fusarium coeruleum* at pH 4·0-9·6, *Scopulariopsis brevicaulis* at pH 6·5-7, *Gloeosporium psidii* at pH 6·0, respectively. The two *Gloeosporium* spp. studied here resemble the above microorganisms in supporting slight growth in the slightly acid medium (pH 6·0). The results of the present study clearly reveal that pH has a marked bearing on the utilization of KNO_2 by these three anthracnose fungi. Their excellent growth at pH 8-9, and its absence (or very poor growth) on the acid side is in conformity with the generally accepted view that nitrites are toxic in the acid medium while they are not so on the alkaline side. The toxicity on the acid side is due to the presence of free nitrous acid in the medium (Cochrane, 1950 and Cochrane and Conn, 1950, and Wolf, 1947), which produces a destructive effect on the proteins and amino acids of fungal cells (Lilly and Barnett, 1951 and Foster, 1949). The growth of *G. psidii* and *G. piperatum* at pH 6, though quite poor is interesting. At the optimum pH of 8-9, these anthranose fungi yielded almost as much growth as they did with KNO_3 at the optimum pH of 5-6. It is thus apparent that fungi which utilize nitrate as the source of nitrogen can equally utilize nitrite, provided the medium is made suitably alkaline.

Thind and Duggal, 1957, showed best growth of *Colletotrichum gloeosporoides* at pH 8 with KNO_2, which was found to be equal or slightly more than that produced with KNO_3 at the same pH. Thus the pathogens studied in this paper also resemble *C. gloeosporoides* in supporting good growth with KNO_2 (M/20 per litre) at pH 8-9.

The concentration of KNO_2 used in the present investigation was very high (8·41 gm. per litre of the medium which is equal to M/10). It is not known whether utilization of KNO_2 at such strong concentrations has been reported before for fungi, though it is well known for bacteria (Thimann, 1955).

Most of the amino acids tested, served as a good source of nitrogen for the growth of these fungi. All the three pathogens made a poor growth with l-cystine and in this respect resemble other fungi investigated by Steinberg, 1942, Leben and Keitt, 1948, Pelletier and Keitt, 1954, Wolf, 1955, and Thind and Randhawa, 1957. However, Ajello, 1948, has found good growth of *Polychytrium aggregatum* with this amino acid.

These fungi made good growth with aspartic and glutamic acids, which have generally been reported as the best sources of nitrogen by numerous investigators for many fungi. However, Tandon and Grewal, 1956, Pawar and Patel, 1957, have observed poor growth of *Gloeosporium papayae*, *G. musarum* and *Colletotrichum papayae* with aspartic acid and *Alternaria ricini* and *Phomopsis vexans* with glutamic acid respectively.

These fungi gave good growth with urea as is also observed with *G. payayae*, *G. musarum* and *C. papayae* (Tandon and Grewal, 1956), *Alternaria ricini* (Pawar and Patel, 1957), *Pythium* spp. (Saksena, 1940), *Endoconidiophora moniliformis* (Gordon, 1950), *C. capsici* (Thind and Randhawa, 1957). However, Uppal *et al*, 1933, and Srivastava, 1951, have observed poor growth of *Alternaria* and *Curvularia lunata* respectively with this amide.

These pathogens gave good growth with asparagine and in this respect resemble other fungi investigated by Saksena *et al*, 1952, Srivastava, 1951, Tandon and Grewal, 1956, and Patel *et al*, 1950.

The above study clearly reveals that the three fungi can utilize nitrate nitrogen, ammonium nitrogen and organic nitrogen, but are unable to fix the atmospheric nitrogen, and thus fall under the second group of Robbins's classification of fungi based on the nitrogen requirements, (Robbins, 1937).

SUMMARY

A comparative study on the effect of different nitrogenous compounds on the growth of three anthracnose fungi, *G. psidii* (from guava), *G. piperatum* (from chillies), *Colletotrichum* sp. (from citrus) was carried. These fungi produced good growth with almost all the inorganic compounds tested except nitrites. *Colletotrichum* sp. did not give any growth at pH 6 with nitrites, but *G. psidii* and *G. piperatum* gave some growth at this pH. All these pathogens utilized nitrites (M/10 per litre) excellently in the alkaline medium (pH 8·9). These fungi utilized nitrate nitrogen, ammonium nitrogen and organic nitrogen but failed to grow with atmospheric nitrogen. Almost all the amino acids gave fair to good growth with these fungi except l-cystine. Poor growth, however, occurred with *G. psidii* only on glycine, dl-leucine, l-leucine,-dl-alanine and dl-norvaline; with *Colletotrichum* sp. on dl-norvaline, dl-lysine monohydrochloride and l-tryptophane.

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*THE MORPHOLOGY AND HISTOLOGY OF THE ALIMENTARY TRACT OF HILSA ILISHA (HAMILTON)

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INTRODUCTION

The morphology and histology of the alimentary canal have received the attention of ichthyologists a long time ago. Oppel (1886), Sullivan (1907) and Biedermann (1911) have given exhaustive historical reviews of the work done till that time. Jacobshagen (1911, 1913, 1915, 1937) has later on done important and valuable work on different groups of fishes. Gulland (1898) and Greene (1912, 1914) carried out their works on Salmonidae, the latter, more particularly has dealt with it in fairly good detail. Dawes (1929) has worked out the alimentary canal of a single species belonging to the Pleuronectidae and he too has given a fairly good historical review. Blake (1930, 1936) studied the comparative histology of the digestive tube of *Centropristes striatus* and *Prionotus carolinus*. Works of Rogick (1931), Curry (1939), Sarbahi (1940), Al-Hussaini (1949) and Girgis (1952), are directly concerned with cyprinids. Ishida (1935), Ghazzawi (1933, 1935) and Pillay (1953) have worked on the alimentary canal of Mugilidae. In the recent years exhaustive work on different groups of fishes has been done by Al-Hussaini (1945, 1946, 1947, 1949, 1953). Futhermore, in India, Mohsin (1944-46) worked out the alimentary canal of *Anabas testudineus*, Ahsan ul-Islam (1951) that of *Rita rita Cirrhina mrigala* and *Ophicephalus gachua*. Rahimullah (1935, 1943, 1945, 1947 and 1948) has worked out the morphology, histology and probable functions of the pyloric caeca in Indian fishes and has also discussed its homology. He has also given a good historical account of the subject. Al-Hussaini (1946-47) has also fully discussed it. Commendable work on the pancrease of different teleostean fishes has been done by Woodland (1911), Hill (1926), Crystal (1946) and various others. Studies on the food and feeding habits of fishes in India have been attempted by Hornell and Naidu (1923), Job (1940, 1941), Menon (1942), Mukerji *et al* (1946, 1949), Bapat and Bal (1950), and Das and Moitra (1955, 1956*a* and 1956*b*).

It becomes thus clear that works on all the branches of morphology, histology of the alimentary canal, food and feeding habits have been carried out in most of the groups of teleostean fishes but, as far as, the author is aware, Clupeidae, one of the most primitive families of Teleostei, has escaped the attention of workers.† Casual reference regarding the work on herring by Stirling (1884), on Salmon by Greene (1912) and on gizzard shad, *Derosoma capedianum* by Weir and Churchill (1915) has been made by Al Hussaini (1947, 1949). It is regreted that the author could not consult the originals of these works because of their non-availability. The present work was, therefore, taken up to study the morphology and histology of the alimentary canal in the Indian shad, *Hilsa ilisha*, which is one of the most important food fishes of India.

* A part of the thesis approved for the degree of Doctor of Philosophy of Allahabad University in 1957.

†Quite recently the present author had an opportunity to go through an account of the morphology and histology of the alimentary tract of a plankton-feeder Gadusia chapra (Kapoor 1958).

MATERIAL AND METHODS

The fish for the present study were collected from the rivers Ganga and Jamuna covering a radius of about forty miles round Allahabad. They were caught alive and the various parts of alimentary canal were fixed in Bouin's picro-formal-acetic fluid. For the study of the histology of the buccal cavity the entire head of the specimens measuring 30-40 mm. were decalcified after fixation. The material was embedded in paraffin and serial sections were cut at 6-8 microns in thickness. Dalafield's haematoxylin counter stained with eosin gave excellent results. For the study of nerve endings in buccal cavity Schandinni's fluid was used as fixative.

The study of food and feeding habits was taken up with a view to study the nutritional cycle and its correlation with the breeding habits. For this purpose numerous specimens were cut open in the field itself and the whole viscera fixed in 5% formaldehyde. The stages of maturity of the specimens were also noted. Juveniles were also generally cut open in the field and the entire fish fixed in 5% formaldehyde.

The extent of the feed was determined by the degree of distension of the stomach and the amount of food it contained. In spite of the limitations of such an estimate this was the only practicable method that could be employed in the present study. The condition of feed was classified as (*i*) gorged (stomach swollen and expanded particularly the cardiac stomach), (*ii*) Full, (*iii*) $\frac{3}{4}$ full (*iv*) $\frac{1}{2}$ full, (*v*) Food in traces (*vi*) Empty.

The gut contents were analysed in the following manner.

At first the volume of entire stomach content was determined by displacement method. The contents were then made to a known volume by adding 5% formaldehyde. This known volume was made homogenous by shaking slightly and a drop equivalent to ·1 cc was examined to determine the percentage of the different items of the feed including sand and decayed organic matter. The percentage was determined by eye estimation. After examination the sample was again dropped into the stock. This process was repeated thrice. Monthly average for different items in relation to the sex has been noted.

A good percentage of specimens examined had their stomachs empty. Many fishes are in the habit of throwing up their last meal when captured (Affalo and Martson, 1904). Ogilvie (1927) found that the stock of capture did not induce the postlarval herring to throw out their food. The author agrees with Ogilvie (1927), Job (1940) and Pillay (1953) in this respect which goes against the observations of Affalo and Martson (1904).

ACKNOWLEDGEMENTS

I am deeply indebted to Dr. S. K. Dutta, for guiding me in this work and to Prof. A. H. Al-Hussaini for going through the manuscript. I am particularly thankful to Dr. P. N. Srivastava for his valuable suggestions and Dr. D. N. Verma for help in publication. To Mr. Ramapati Rai I must also express my thanks for his assistance in preparing the diagrams.

Finally, I have to gratefully acknowledge the financial aid received from the Council of Scientific and Industrial Reasearch.

Gross Anatomy of the Alimentary Canal :

The alimentary canal of *Hilsa ilisha* (Hamilton) consists of buccal cavity, pharynx, oesophagus stomach, small intestine (duodenum and ileum) and large intestine (rectum).

The buccal cavity is laterally compressed and the gape of the mouth is bounded above by the maxila and premaxilla which are movably articulated with each other. The dentary forms the boundry of the mouth on the lower side. The buccal cavity is devoid of teeth as a result of which the food materials are swallowed in as a whole. The pharynx represents a small narrow area between the buccal cavity and the oesophagus. The latter is also very short and communicates with the cardiac stomach. On splitting open, numerous raised patches of different shapes can be marked at the posterior extremity of the oesophagus. These patches serve the function of valves and here the muscles are arranged in distinct bundles. The cardiac stomach is J-shaped and at its posterior extremity opens the pneumatic duct which connects the stomach with the air bladder. The pyloric stomach is globular in appearance with a highly developed muscular layer; and it is here that the food material is ground. The pyloric stomach is connected with the duodenum.

Of special interest are the innumerable clusters or tufts of small, extensively distributed pyloric caeca which open into the duodenum. They are crowded immediately behind the pylorus and extend completely over the duodenum on the ventral and lateral sides alone (figs. 1, 3 Plate I). They are not found over the dorsal surface. The caeca are larger in size near the pyloric end and form a sort of cap over the head of the pylorus. All the clusters are composed of 10-12 small diverticula. In the posterior region the tufts are smaller in size and consist of only 6-8 diverticula. All the clusters open directly into the duodenum (Fig. 3 Plate I). There are in all about 3351 caeca arranged in 313 clusters.

The small intestine is a coiled tube and its various loops can be well marked in fig. 1 Plate I. The rectum is not very much distinct from that of the intestine.

Food and Feeding Habits :

Fishes, both juvenile and adults, had been collected in hundreds and the contents of the stomach examined. Adult fishes were caught throughout the year whereas the juveniles were collected from the last week of April to the last week of June. The feeding cycle of adults, both male and female, and juveniles had been worked out separately. The following table will give an idea of food and its percentage in adults throughout the year.

Month	Sex	Condition of stomach	% of food	Remarks
January	Male	94·5% Empty	Cladocerans 5%	Starvation period
		5·5% Food in traces only	Decayed organic matter	
	Female	95·0% Empty 5·0% Food in traces only	95%	

Month	Sex	Condition of stomach	% of food	Remarks
February	Male	82·0% Empty	Rotifer 67% and eggs	Semi Starvation period for male
		9·0% Food in traces	Ulothrix 8%	
			Cladocerans 11·8%	
		9·0% Gorged with food	Spirogyra ·4%	Complete
	Female	100·0% Empty	Decayed organic matter 20%	Starvation period for female
March	Male	55% Empty	Cladocerans 15·4%	
		17·5% Food in traces	Lynbia 0·7%	
		10% Quarter full	Spirogyra 39·0%	Maximum feeding period
		12·5% Hall Full	Synedra 0·5%	
		5% Gorged with food	young prawn 0·3%	
	Female	56·3% Empty	Decayed organic matter 44%	
		15·6% Food in traces		
		15·6% Quarter full		
		3·1% Half full		
		3·1% Three-fourths full		
		6·3% Gorged with food		
April	Male	70·9% Empty	Cladocerans 55%	
		23·6% Food in traces	Lynbia 5%	Average feeding period
		6% Half full	Spirogyra 2%	
		5·4% Three-fourths full	Sand & Mud 6%	
	Female	66·6% Empty	D. O. matter 32%	
		6·6% Traces	(Decayed organic matter)	
		20·0% Quarter full		
		6·6% Half full		

Month	Sex	Condition of stomach	% of food	Remarks
May	Male	78.9% Empty 13·1% Food in traces 2·6% Half full 2·6% Three-fourths full 2·6% Gorged with food	Cladocerans 20·8% Ulothrix 1% Synedra 2% Sand & Mud 24% Decayed organic matter 54%	Feeding decreases
	Female	70·3% Empty 25·9% Food in traces 3·7% Quarter full		
June	Male	76% Empty 23·8% Food in traces	Cladocerans 15% Rotifer 25%	Semi starvation period
	Female	87·5% Empty 12·5% Quarter full	Spirogyra 5% Sand & mud 5% Decayed organic matter 50%	
July	Male	85·7% Empty 14·3% Food in traces	Cladocerans 20% Rotifer 20%	Semi starvation period.
	Famale	75% Empty 25% Food in traces	Sand 10% Decayed organic matter 50%	
August	Male	55·5% Empty 18·5% Food in traces 18·5% Quarter full 7·4% Gorged with food	Cladocerans 46% Melocera 14% Decayed organic matter 40%	
	Female	37·5% Empty 12·5% Food in traces 12·5% Quarter full 12·5% Three fourths full 25·0% Gorged with food.		Maximum feeding period

Month	Sex	Condition of stomach	% of food	Remarks
Sept.	Female	64·2% Empty	Cladocerans 32 %	
		21·4% Food in traces	Decayed organic matter 48 %	Maximum feeding period
		14·2% Three fourths full	Melocera 16%	
	Male	40·9% Empty		
		27·2% Food in traces		
		9·0% Half full		
		22·7% Three fourths full		
Oct.	Male	68% Empty	Cladocerans 42 %	
		22·7% Food in traces	Decayed matter 42%	Feeding decreases
		4·5% ¼ full	Rotifer 2%	
		4·5% Half full		
	Female	56·0% Empty	Sand 4%	
		28·0% Food in traces		
		9·3% ¾ full	Melocera 9%	
		6·2% Gorged with food		
Nov.	Male	90% Empty	Cladocerans 35%	Starvation period.
		10% Food in traces	Rotifer 2%	
	Female	93·6% Empty	Sand 16%	
		6·4% Food in traces	Decayed matter 46%	
			Melocera 5 %	
Dec.	Male	95·5% Empty	Cladocerans 8 %	
		4·5% Food in traces	Rotifer 1·6 %	Starvation period
			Spacerocyst 1%	
	Female	95·5% Empty	Synedra 2·2%	
		4·5% Food in traces	Decayed matter 71·6 %	
			Sand 15·3%	

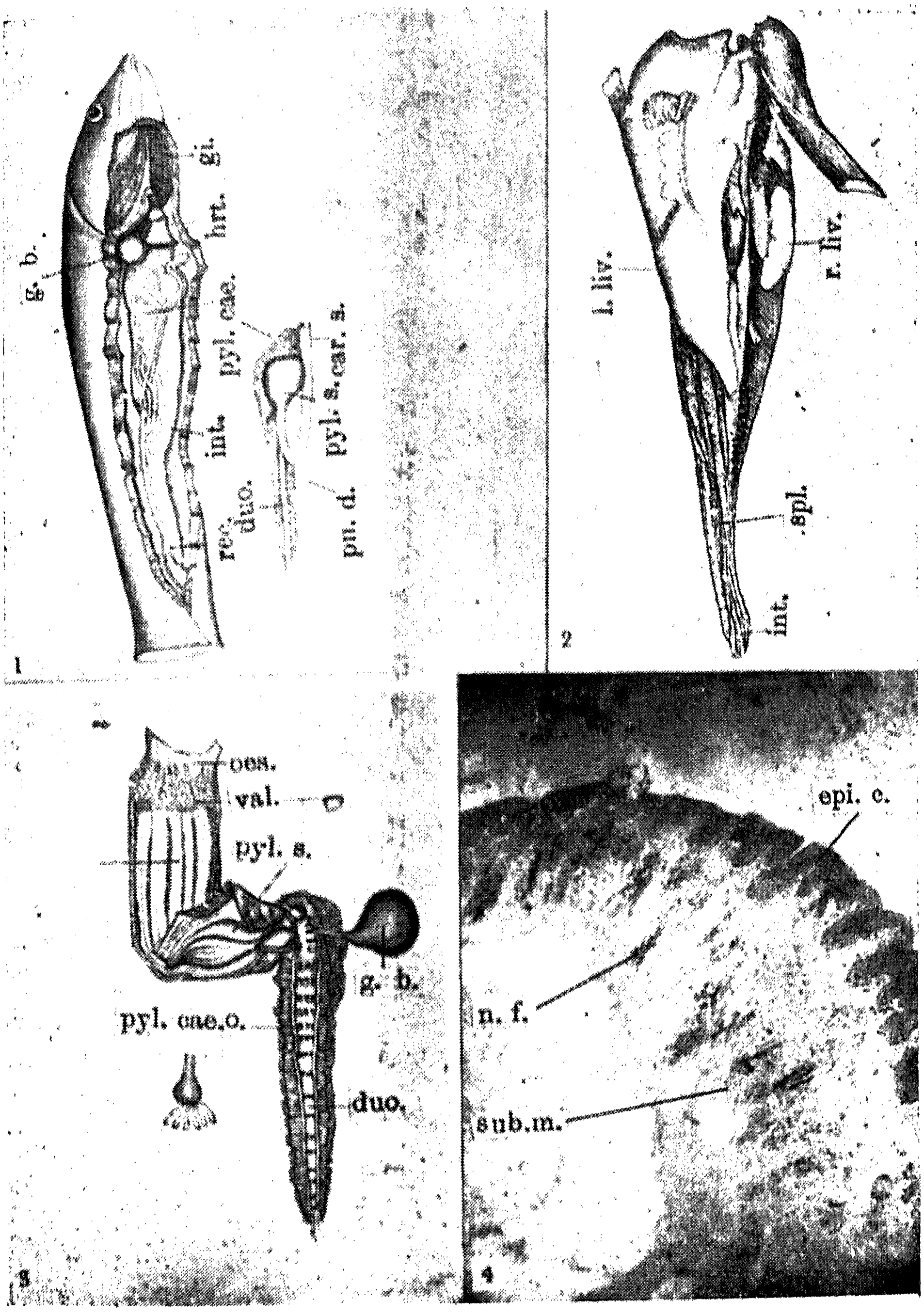
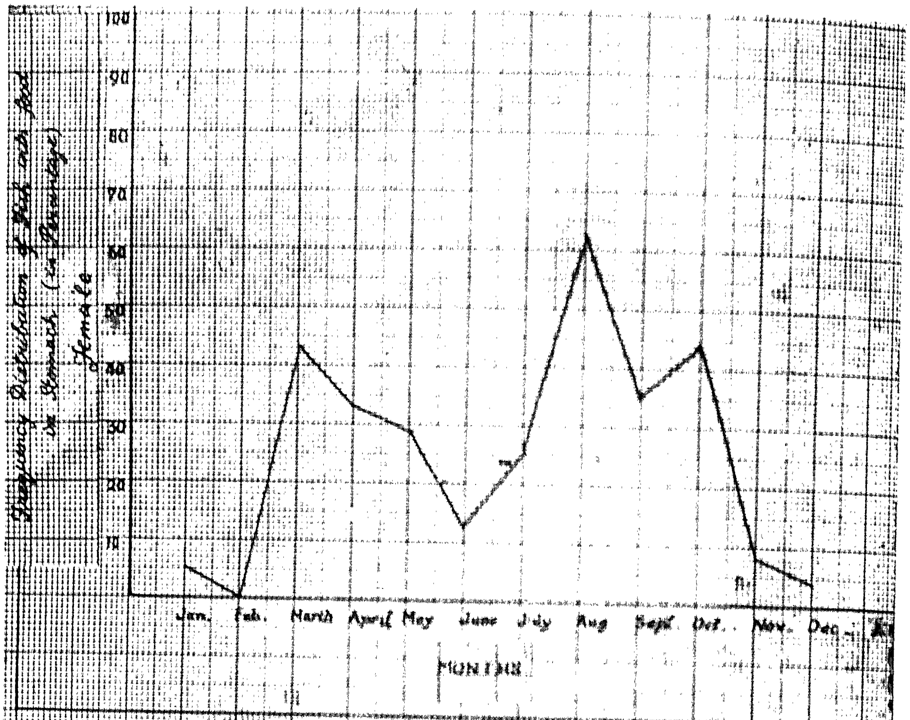


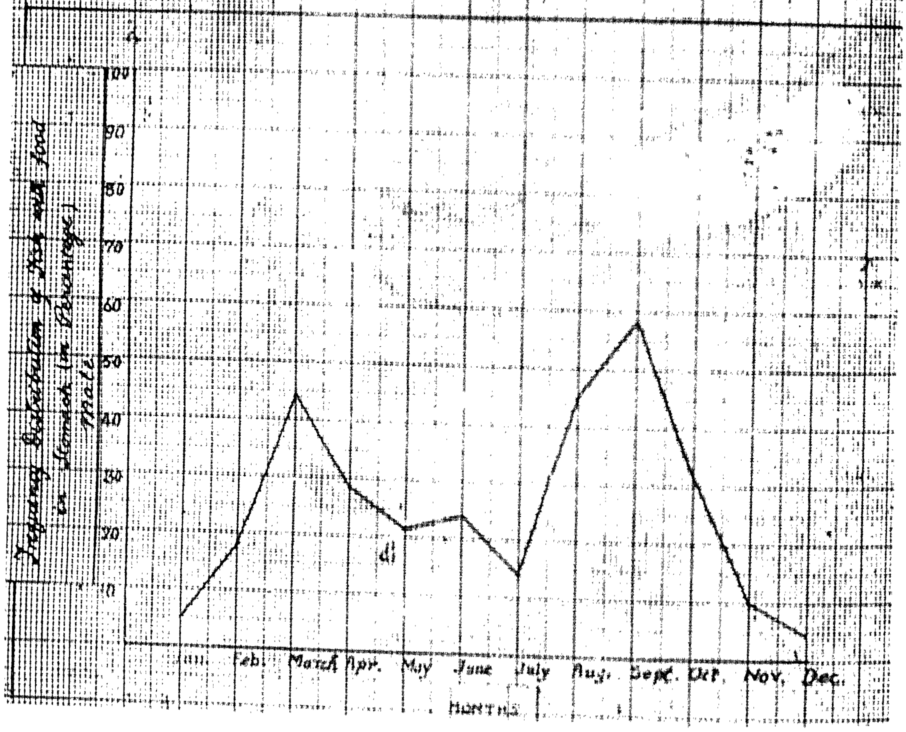
Plate I, Fig. 1. Fish dissected latero-Ventrally to show the general disposition of the alimentary canal.

Fig. 2. Alimentary canal taken out with the liver to show the liver from the left side.

Fig. 3. A part of the alimentary canal (Oesophagus, cardiac stomach, pyloric stomach, and duodenum) split open to show the general ridges of the alimentary canal and the openings of the pyloric Caeca and the bile duct. A single bunch of pyloric caeca has also been shown separately.

Fig. 4. Photomicrograph of the T. S. of the lower jaw (Anterior region) to show the rich supply of the nerve fibre in the sub-mucosa (Low power).


Frequency Distribution of Fish with food
in Stomach (in Percentage)
Female
100
90
80
70
60
50
40
30
20
10
Jan.
Feb.
March
April
May
June
July
Aug
Sept.
Oct.
Nov.
Dec.
MONTHS


Frequency Distribution of Fish with food
in Stomach (in Percentage)
Male
100
90
80
70
60
50
40
30
20
10
Feb.
March
Apr.
May
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
MONTHS

Juveniles which were collected mostly in May and June are voracious feeders and their food consisted of Rotifer 8·9% ; Ulothrix 10·0% ; Spirogyra 10·0% ; Pediastrum 3·0% ; Aplanocaspa 1·1% ; Phoronidium 1·4% ; Cladocerans 2·5% ; Melosera 3·4% ; Decayed organic matter 31% and Sand 34%.

A close study of the fore-going table conclusively indicates that there are two periods of maximum feeding alternating with a starvation or semi-starvation period both in case of males and females. The maximum feeding periods in case of males are March and September, whereas in case of females it is March and August (Graphs 1 and 2). Adult *Hilsa* is a surface-feeder and is not ordinarily found to eat at depths below twelve feet. This fact is also corraborated with the study of their food. In the months of March, August and September when they actively feed, sand particles are not found at all in the stomach. In other months sand appears in the stomach with varying percentages. This appearance of sand is not because of their actual feeding habits but because of the fact that fishes after spawning are completely exhausted and are forced to go down to the bed of the river so that they may not be affected by the swift currents of the river. Thus it is because of their exhaustion that they are forced to feed at the bottom of the river. It may be mentioned here that Allahabad *Hilsa* has got two spawning periods *i.e.* September to November and March to April and the spawning reaches its peak in November and April respectively.

Southwell and Prasad (1918) had reported that *Hilsa* that migrate up the river Hoogly for spawning do not feed. Allahabad *Hilsa* migrates up to breed in August-September and March-April and under both these circumstances, Hilsa was found to be actively feeding. This observation of the author is in agreement with that of Hora and Nair (1940) who have reported "Though sexually mature, *Hilsa* was feeding in the river near Allahabad."

The Indian representative at the Indo-Pacific Fisheries Council Bhimachar (1955), had reported that the intensity of feeding increases in the spent *Hilsa* in the river Hoogly, but just contrary to it, the feeding of spent *Hilsa* decreases immediately at Allahabad.

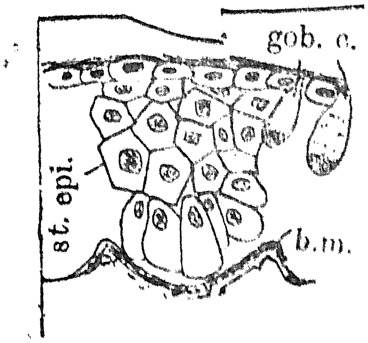
Contrary to the adult *Hilsa*, their juveniles are bottom-feeders because a large quantity of sand is found mixed up with their food. During experiments for the artificial propagation of *Hilsa* at Calcutta and Madras it has been definitely ascertained that after fertilisation the eggs sink deep into the water and begin to float or rest near the bottom. So the young *Hilsa* generally flow down in the rivers in order to avoid the swift currents and feed at the bottom.

HISTOLOGY

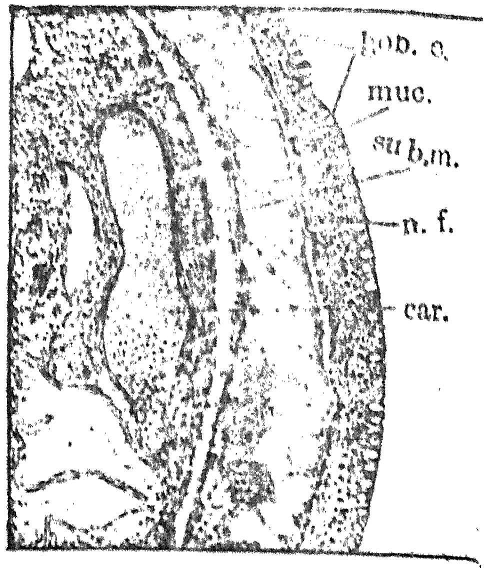
The portions of the alimentary canal studied were from the buccal cavity, pharynx, oesophagus, stomach, intestine along with the rectum.

Buccal Cavity :

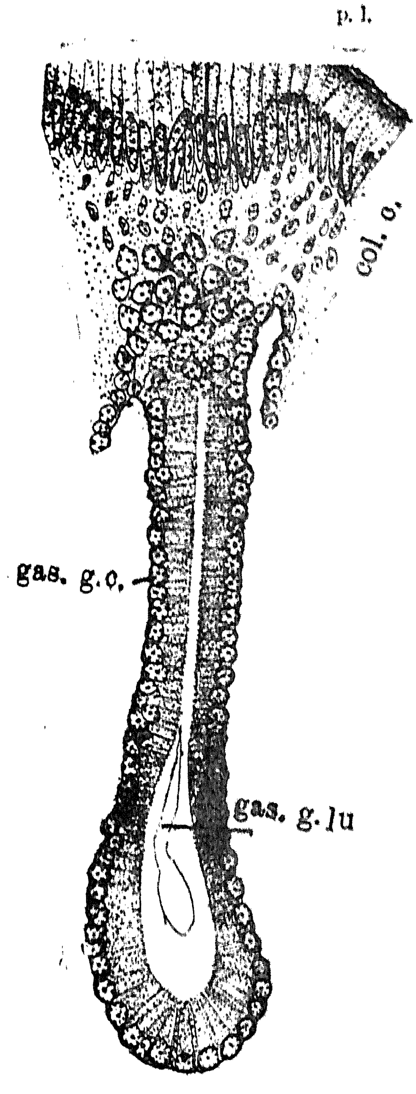
The lining of the buccal cavity consists of mucosa and sub-mucosa separated by a thin basement membrane. The mucosa consists of ordinary epidermal cells which are cubical and oval in shape on the surface but the cells lying on the basement membrane are somewhat rectangular with rounded nuclei. Goblet cells, at the tip of the buccal cavity, are sparingly present just below the surface, some of which can be marked to open to the outside. Buccal cavity is lined throughout by a stratified epithelium (Text Fig. 1). Sub-mucosa is thicker than the mucosa which consists of compactly arranged connective tissue fibres. Inside the sub-mucosa there is a very rich supply of nerve fibres (Fig. 4 Plate I).



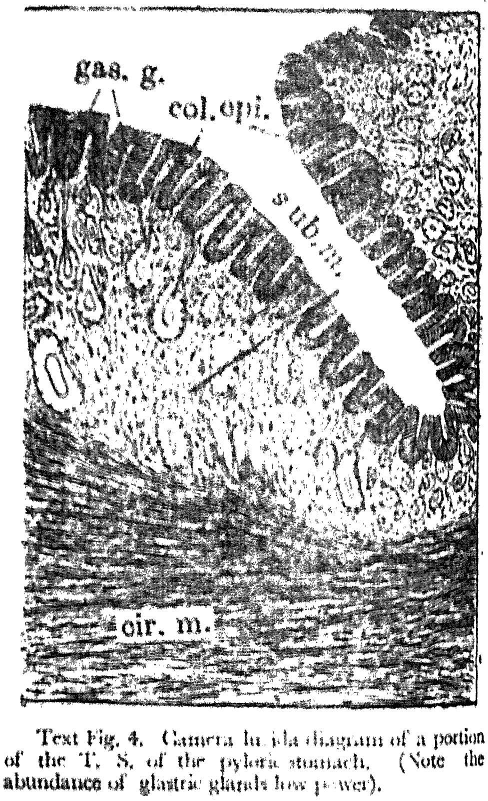
Text Fig. 1. Camera lucida diagram of T. S. of the upper jaw passing through the buccal cavity (Highly magnified).



Text Fig. 2. Camera lucida diagram of the T. S. of the epithelium in the region of the gill-rackers (under low power with 15x eye piece).



Text Fig. 3. Camera lucida diagram of a portion of the T. S. passing through pyloric stomach showing a single gastric gland and columnar epithelium celles (Highly magnified, drawn under 6x eye, piece and oil mmersion objective).



Text Fig. 4. Camera lucida diagram of a portion of the T. S. of the pyloric stomach. (Note the abundance of glastric glands low power).

The tongue is supported by a cartilage. The epithelium over the tongue is very thin. The goblet cells considerably increase in number in the mucosa, whereas there is nothing particular about the nerve cells in the sub-mucosa (Fig. 5 Plate II).

In the epithelium behind the tongue region again the goblet cells are reduced in number but are even then more than at the tip of the buccal cavity. The nerve fibres reappear in abundance (Fig. 6 Plate II). The epidermal cells over the basement membrane in this region are very clearly rectangular with round nuclei.

In the epithelium of the upper jaw, the occurrence of the goblet cells is average, that is, it is neither abundant nor scarce. Nerve fibre concentrations are conspicuous by their absence from the sub-mucosa (Fig. 7 Plate II). The general surface of the mucosal epithelium receives gustatory sense and the same is passed to the brain by nerve.

Pharynx :

In the pharyngeal region the gill-rackers are setose, long and slender and are closely set on the branchial arches in a sieve-like fashion. The gill-rackers are very well adopted for straining microscopic plankton from the water. In appearance they recall the baleen plates of the whalebone whales which are also adapted for straining minute organisms from water.

In the region of the gill-rackers the mucosa is composed of stratified epithelium and goblet cells. It is surprising how Kapoor '54 denies the presence of goblet cells in this region. The goblet cells, are arranged in a continuous row over the surface of the mucosa. The nerve fibres are also arranged in a row just below the basement membrane (Text fig. 2).

Kapoor (1954) has studied the pharyngeal organ of *Hilsa ilisha*. The pharyngeal organ can be seen after removing the first three pairs of gill arches. It consists of a pair of curved blind diverticula of the pharynx. Each diverticulum has a canal passage and a blind sac. The histology of the canal passage is similar to that of the pharynx and he concludes that the pharyngeal organ is simply a diverticulum of the pharynx and that it has no respiratory function.

The wall of the pharynx is composed of four coats, which are serosa, muscularis, sub-mucosa and mucosa. Goblet cells are abundant in the mucosa which is thrown into folds in this region. The supply of nerve fibres are also very rich but they are not arranged regularly just below the basement membrane. The sub-mucosa is very well developed. It is compact and numerous muscle bundles of striated nature are scattered throughout (Fig. 8 Plate II).

Oesophagus :

The pharynx merges insensibly into the oesophagus. The mucous secreting goblet cells considerably increase in number. The mucosal folds which are thicker in pharynx become gradually thin and high in oesophagus. The epithelium changes from the stratified type into columnar type. The arrangement of nerve fibres in the mucosa is the same as in the pharynx (fig. 9 Plate III). Such an abundant supply of nerve fibres in the sub-mucosa in buccal cavity, pharynx and oesophagus has not yet been described in any other fish.

Cardiac Stomach :

Fundamentally the cardiac stomach is also composed of the same four layers as those of the oesophagus. The histological structures of the oesophagus do

not abruptly end posteriorly but they persist for a short distance and some new features are added up gradually.

The serosa is thin and consists of a layer of flattened peritonial cells except in places where blood vessels and surrounding connective tissues are found. Muscularis layer consists of longitudinal and circular muscles which are separated by connective tissue. The sub-mucosa and mucosa are separated by the tunica propria which is a well defined unstriated layer of connective tissue, somewhat similar to that of the sub-mucosa. The epithelium of the mucosa consists of typical columnar cells, which is occasionally turned inwards into crypts (fig. 10 Plate III). These cells are large, rectangular with oval nuclei. The gastric glands are present surrounding the crypts. Each crypt receives the openings of the gastric secretary glands. The nuclei of the gastric secreting cells are minute as compared to that of the columnar cells. The gastric glands are numerous in the posterior cardiac stomach and are scarce in the anterior portion. Goblet cells are completely absent.

Pyloric Stomach :

In the pyloric stomach the cellular layers are the same as those of cardiac stomach. The muscularis layer is very highly developed and is responsible for the grinding of the food. The thick circular layer of the striated muscles constitutes about two thirds of the entire thickness of the wall. In this region the mucosa forms deep folds generally arranged in longitudinal fashion and forms numerous crypts. The epithelium of the mucosa is of the slender columnar type. The cells are larger and slender with larger nuclei. There is a protecting layer of non-cellular material lying upon the columnar cells of mucosa (Text Fig. 3). Just below the columnar layer, gastric glands are profusely and evenly distributed throughout in the sub-mucosa (Text Fig. 4). These glands with tubular neck consist of cells arranged in a circular row with lumen in the middle. The cells are elongated with rounded nuclei situated at the peripheral end. Numerous of these glands can be seen opening into the crypts. The presence of gastric glands in such abundance has not been recorded from the pyloric stomach of any other fish. There is nothing particular about the sub-mucous layer. The tunica propria is not very distinctly developed. Goblet cells are completely absent.

Duodenum :

This is the part of the intestine which begins at the pyloric valve which marks the limit of the pyloric stomach. It is here that the numerous intestinal diverticula or pyloric caeca open (fig. 3 Plate I). The histological structure of the duodenum is more or less similar to that of the pyloric caeca. The structural constituents of duodenum is not simple because its wall is broken up, by the origin of numerous caeca. Its complete structure can be studied from its dorsal wall because the pyloric caeca arise only from the ventral and lateral surfaces. Its wall consist of serosa, muscularis layer both circular and longitudinal, sub-mucosa a d mucosa. Tunica propria divides the sub-mucosa and mucosa. The inner epithelium has got long epithelial cells with big oval nuclei. Goblet cells are scarce.

Pyloric Caeca :

The clustering of caeca on the ventral and lateral sides of the dudoenum of *Hilia ilisha* is very interesting and characteristic (fig. 1 & 3 Plate I). Histologically

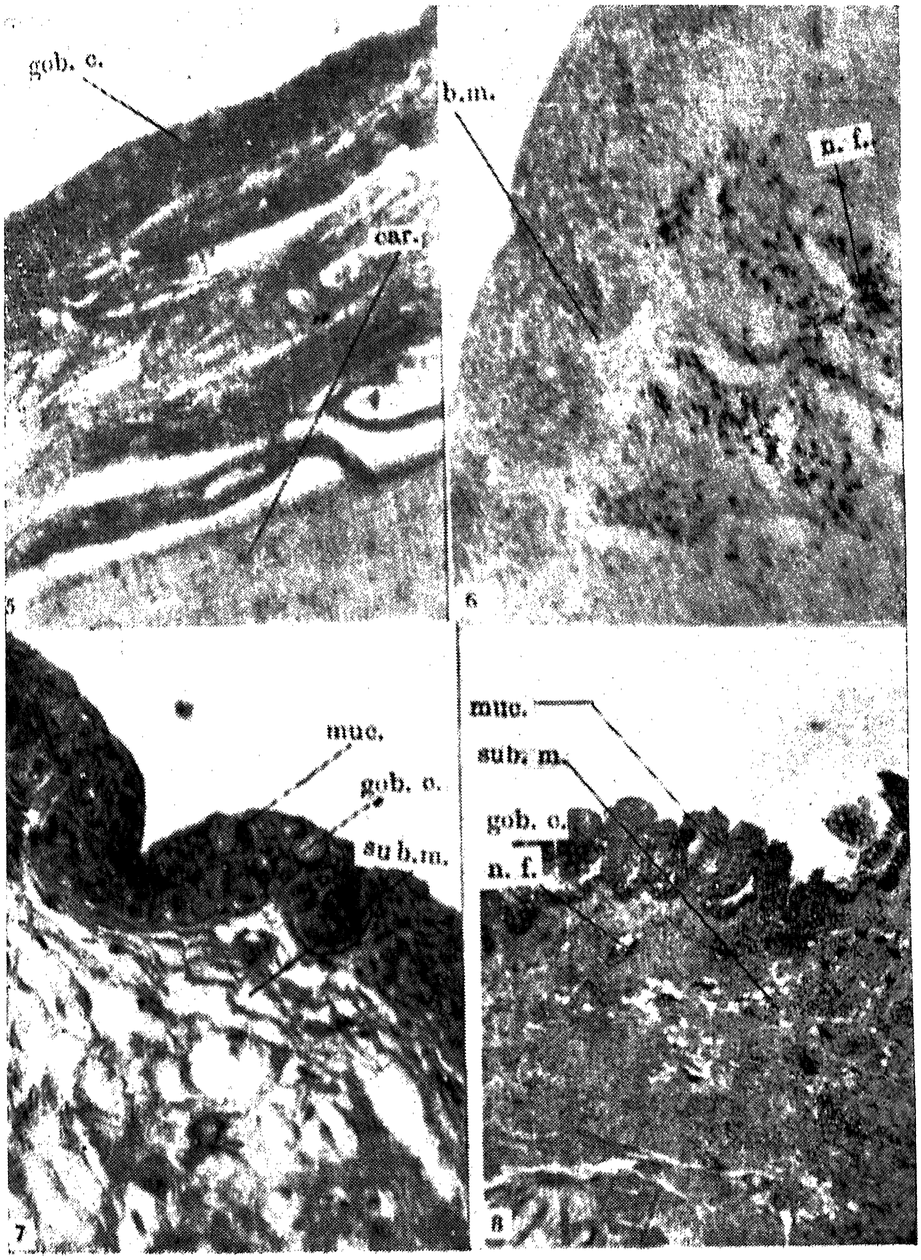


Plate II, Fig. 5. Photomicrograph of the T. S. of the buccal cavity (tongue region). Nerve fibres are absent (High power).

Fig. 6. Photomicrograph of the T. S. of the buccal cavity behind the tongue region. Nerve fibres are in abundance (High power).

Fig. 7. Photomicrograph of the T. S. of the upper jaw epithelium. Nerve fibres are absent (High power).

Fig. 8. Photomicrograph of the T. S. of the pharynx (Low power).

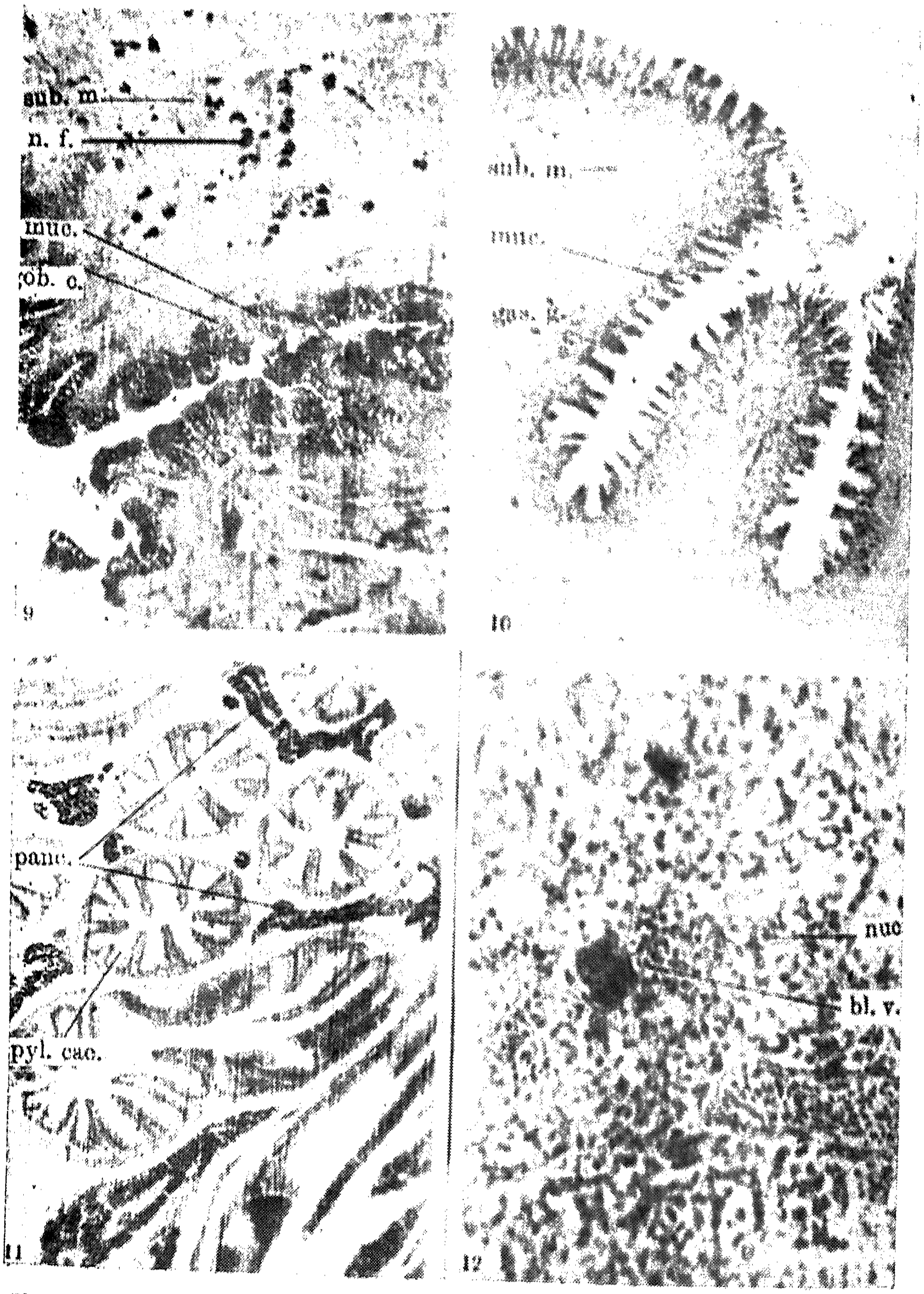


Plate III, Fig. 9. Photomicrograph of the T. S. of the oesophagus to show the intensity of the nerve fibres and the goblet cells. (Low power).

Fig. 10. Photomicrographs of the T. S. of the cardiac stomach (posterior region) to show the presence of gastric glands (Low power).

11. Photomicrograph of the T. S. showing the pyloric caeca and pancreas in low power.

Fig. 12. Photomicrograph of the T. S. of spleen (High power).

the structure of pyloric caeca does not show any marked difference from that of the intestine which it resembles more than with the duodenum (fig. 11 Plate III). The serosa is extremely thin. The longitudinal and circular muscle fibres are also thin and do not show any particular features. The sub-mucosa consists of more or less loose areolar connective tissue. Numerous fine blood vessels and nurve fibres are also embedded in it. It is separated from the mucosa by a thin layer of tunica propria. The mucosa is thick and is considerably folded. Rahimullah (1945) termed these fold as caecal villi. The cells of the inner epithelium are slender columnar with oval nuclei. Goblet cells are scarse in juvenile forms whereas in adults they are present but not in plenty. Num e rous wandering leucocytes can be noticed.

Intestine :

There is nothing particular in the cells of serosa. The muscularis layer of the intestine consists of an outer longitudinal layer and an inner circular layer consisting of striated muscle fibres alone. The blood supply is rich. Occasionally a section will show arterioles entering the intestine from the exterior penetrating to distribute branches in between the two muscular coats and also in the sub-mucosa. The nature of the sub-mucosa is the same as we get in the case of the pyloric caeca. The mucosa is lined throughout by two kinds of cells. (*a*) Columnar cells which are absorptive cells. They are elongated, slender with large oval nuclei and (*b*) Mucous secreting gobelt cells, which are present in abundance. They open into the lumen of the intestine by a narrow neck. They have also got a narrow basal portion in which the nucleus is lodged. Tunica propria, the layer which separates the mucosa with the sub-mucosa, is not very distinct but merges insesibly into the thin sub-mucous connective tissue outside it (Fig. 13 Plate IV and text Fig. 5).

Rectum :

Strictly speaking, there is not much histological difference in the structure of intestine and rectum and the latter consists of serosa, muscularis, sub-mucosa and mucosa. Mucosal folds are complex. Tunica propria is thicker and more distinct than in the region of the intestine. The absorptive columnar cells in the epithelial layer of the mucosa are very scarce, and its place is taken up by the goblet cells. As such ninty percent of the cells of the columnar epithelium of rectum are goblet cells (fig. 14 Plate IV). This clearly indicates that rectum does not take any particular part in the absorption of digested food material.

Li ver :

The liver is divided into two irregular lobes, the one on the left side is about twice that of the right side in volume. Both of the lobes are united with each other just over the place where the pyloric caeca form a sort of cap over the pyloric end of the stomach. The right lobe is further irregularily divided into three parts. These parts particularly cover up the pyloric caeca. A part of the liver also extends towards the posterior part of the abdominal cavity. The left liver lobe more or less covers the loops of the intestine which are located in the anterior part of the abdominal cavity (fig. 2 Plate I).

The gall-bladder is thin-walled, nearly spherical in shape and is situated near the anterior extremity of the duodenum, particularly covered over by the anterior most part of the right liver lobe. From the gall-bladder starts the cystic duct, col-

lects the bile from all the various parts of the liver through the respective hepatic ducts. After joining the hepatic duct the cystic duct is called the bile duct and it opens into the anterior duodenum (fig. 3 Plate I). The bile is yellowish green in colour. There is nothing particular about the histological structure of the liver which is similar to that of any other fish.

Pancreas :

It is a compound racemose gland and can be easily distinguished from that of liver. It consists of large polyhedral cells which are aggregated to form acini (fig. 11 Plate III). The cytoplasm is dense and homogenous. Pancreatic tissue is abundant in *Hilsa ilisha* and it is found in the mesenteries, known as adiposo-pancreatic tissue, which bind all the pyloric caeca together. They are also prominently arranged around the blood vessels of the mesentery.

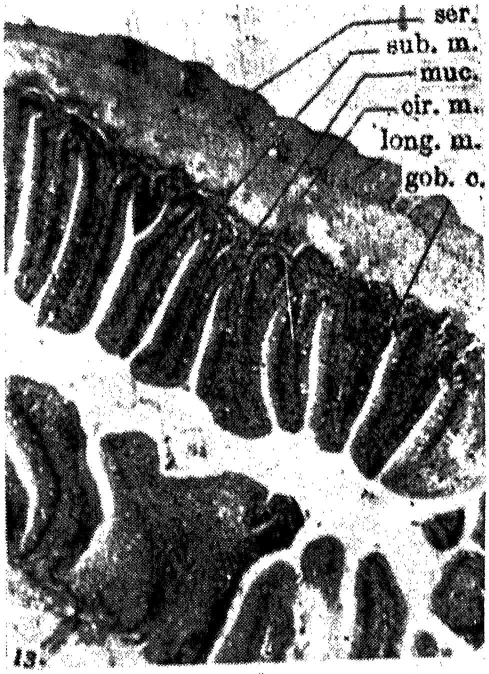
Spleen :

Spleen in case of *Hilsa ilisha* is very well developed. It is dark red in appearance and lies united with the mesentery which unites the various coils of the intestine. Thus coils of the intestine in the posterior abdominal cavity are filled up completely with various lobes of spleen (fig. 2 Plate I). One lobe of the splenic tissue lies anteriorly also, close to the pyloric caeca and the liver. Each lobe of the spleen is formed of various lobules which are composed of a close network of reticular tissue containing flattened and branched cells along with a number of blood corpuscles (fig. 12 Plate III). The nuclei in the cells are very large.

DISCUSSION

The buccal cavity in case of *Hilsa ilisha* (Hamilton) is devoid of teeth and is provided with a tongue supported by cartilage. A large number of teleostean fishes have been reported to have flask-shaped taste buds lying on the evagination of the connective tissue and are connected with nerve fibres which carry a sensation of taste to the brain. In *Hilsa ilisha* they are completely absent. One would naturally assume that they have no gustatory sense and are blind-feeders. But when we study the food of fish it becomes apparent that it does have some sense of selection and is not a blind-feeder. Histological study of the buccal cavity shows that there is a rich abundant supply of nerve fibres just below the mucosal epithelium. Such a nerve supply is present in the pharynx and oesophagus also but is absent from the other parts of the alimentry canal. These concentrations of nerve fibres seem to function as primitive taste buds in *Hilsa ilisha* which is a member of one of the most primitive families of Teleostei. The mucosal epithelium serves as receptor. Apart from Teleostei about the origin of taste buds in more primitive groups of fishes nothing can be said as the author did not come across any account concerning this problem.

In this primitive case of *Hilsa ilisha* the nerve supply has become rich but the taste buds have not yet originated in the mucosal epithelium. These nerve fibres are responsible for carrying the gustatory sense to the brain. In case of more evolved Teleostean fishes certain parts of the mucosal epithelium become modified into taste-buds. The author evidently concludes that *Hilsa ilisha* is not a blind-feeder but it does apply its sense of taste in the selection of food. Recently Srivastava (1953) reported about the occurrence of similar nerve fibre concentrations in *Gadusia chapra* another member of the family clupeidae and emphatically denies the pre-



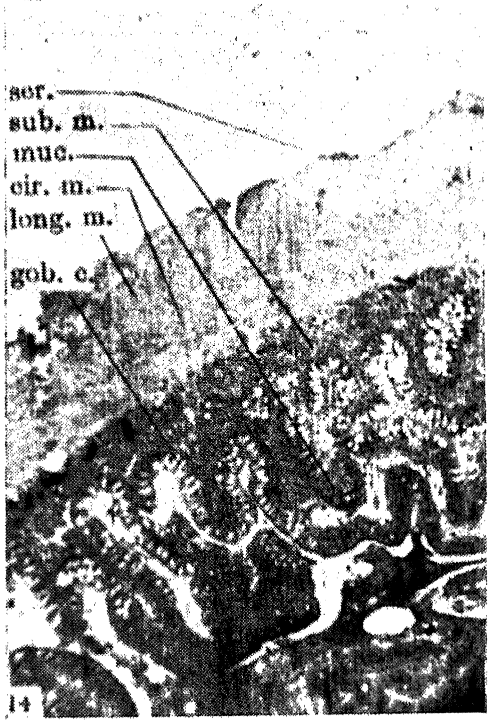
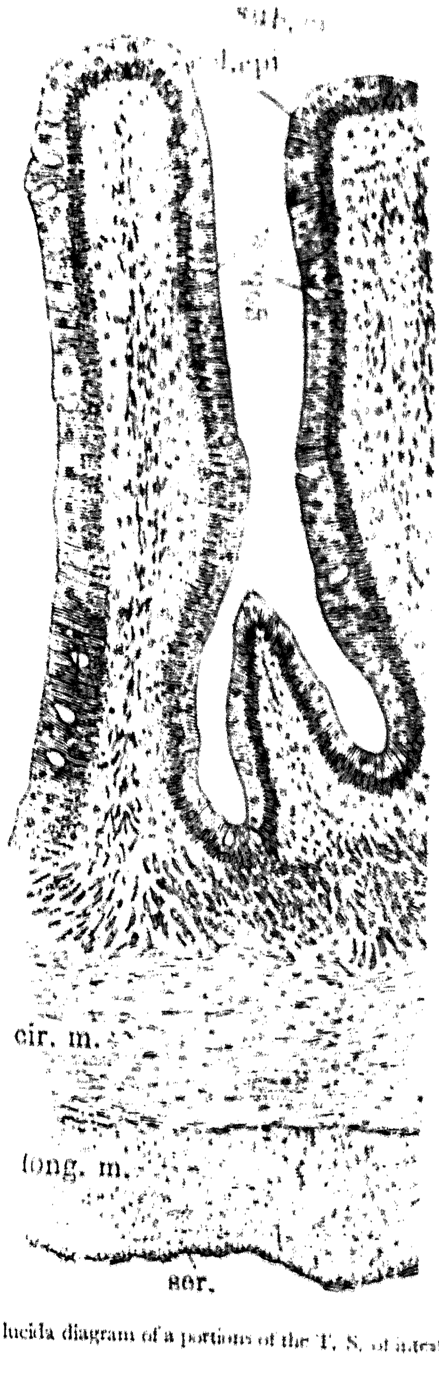


Plate IV, Fig. 13. Photomicrograph of the T. S. of intestine (Low power).
Fig. 14. Photomicrograph of the T. S. of the rectum (Low power).



Text Fig. 5. Camera lucida diagram of a portions of the T. S. of intestine (High power).

sence of taste buds. Kapoor (1958) working on the same fish, however, came across a few small taste buds in the pharyngyal mucose. Srivastava (1958) probably failed to observe them because of their very few number. The co-existence of nerve concentrations and the taste buds indicates that in *Gadusia chapra* the true taste buds are probably originating.

Presence of the concentrations of nerve supply in the sub-mucosa in the buccal cavity, pharynx and oesophagus in *Hilsa ilisha* is very characteristic. It becomes more significant because they indicate the primitive step in the development of taste buds. It also points out that the sense of taste is carried to the brain not only from the buccal cavity but also from the pharynx and oesophagus. Fishes are known where taste-buds are present in the pharynx. Dawes (1929) was the first man to report them in the pharynx of plaice, *Pleuronectes platessa.* Later on, many instances, where the taste buds occur in the pharynx, have been reported by Rogick (1931) in the minnow, Ghazzawi (1935) in grey mullet, Imhof (1935) in Blennidae, Curry (1939) in the common carp, Sarbahi (1940) in the Indian carp and Al-Hussaini (1947) in *Atherina forskali.* But uptil now author has not come across any published account of the occurrence of taste buds in the oesophagus except that of Al-Hussaini (1949) in *Gobio gobio* and *Cyprinus carpio* but even in these cases the taste buds are present only in the anterior oesophageal region. A few taste buds have also been described in case of minnow by Rogick (1931) and in *Labeo horie* by *Girgis* (1952). Hence the presence of such a rich supply of nerve fibres, which carry the gustatory sense to the brain becomes all the more significant and should be regarded as the first step in the origin of taste buds. It also denotes that in *Hilsa ilisha* buccal cavity, pharynx and oesophagus, all are capable of tasting the food.

The buccal cavity is devoid of teeth and the function of the mouth is simply to catch food material and pass it on to the stomach. The mucous producing goblet cells are present in the buccal cavity. They are scarce on the tip but are present in large numbers on the tongue and posterior part of the buccal cavity. This indicates that the function of the tongue is simply to produce mucous so that the food particles may easily slip into the pharynx. Upper jaw epithelium is completely devoid of the net work of nerve supply which shows that it does not take any part in the gustatory sense.

Gill-rackers are characteristically modified in case of *Hilsa ilisha* and are closely set on the branchial arches. These are very well adapted for straining microscopic plankton from the water. In all the fishes which have been reported to be plankton-feeders, the gill-rackers are invariably modified into setiform rakers which are used for straining purposes. Such gill-rakers have been described by Imms (1904) in Polyodon and Seitz (1937) in *Helostoma.* Suyehiro (1934) has compared the carnivorous *Gadus macrocephalus* and plankton feeder, *Theragra chalcogramma* and has shown that the former has coarse, short rakers which cannot collect fine food such as the plankton while the latter has fine ones which can easily retain them. Al-Hussaini (1947) while comparing the alimentary tracts of three types of fishes with different feeding habits namely, Coral-feeder *Scarus sordidus,* the bottom-feeder *Mulloides auriflamma* and plankton-feeder *Atherina forskali,* has also shown that all the fishes described above take in fine particles and gill-rakers are variously modified. But he has definitely shown that in the first two, the function of gill-rakers are to protect the gill filaments from the ill effect of silt material whereas in Atherina they are primarily meant to strain food from the water.

The stomach is divided into two parts—(*a*) the cardiac and (*b*) the pyloric stomach. The histological constituents are almost the same in both parts. The muscularis layer in the cardiac stomach is not so well developed as in the pyloric stomach where it forms almost three-fourths of the whole wall. This muscular

wall is responsible for carrying out the grinding of food. The cardiac stomach in case of *Hilsa ilisha* is mostly used as a reservoir of food whereas the pyloric stomach functions as the true stomach. This becomes conclusively clear if we see the presence and the character of the gastric glands. These glands are very simple in the cardiac stomach whereas in pyloric stomach they are highly developed. Moreover, these glands are very scarce in the anterior part of the cardiac stomach and its number increases gradually towards its posterior region. The sub-mucosa of the pyloric stomach is almost completely occupied by the gastric glands. Certain multicellular glands have been reported in the pyloric stomach by Al-Hussaini and Kholy (1953) in *Tilapia nilotica* and by Berndt (1938) in *Anguilla fluviatilis* but in the latter the glands only guard the orifice of the pyloric stomach. The non-celluler layer lying upon the columnar epithelium of the pyloric stomach protects the mucosa from being injured during the process of grinding of food within the lumen of the stomach. Similar protective layer has been described in the pyloric stomach by Weir and Chruchill (1945) in *Dorosoma cepedianum*, Ishida (1935) in *Mugil cephalus*, Pillay (1953) in *Mugil Tade*, Mahadevan in *Mugil crenilabis* and Kapoor (1958) in *Gadusia chapra*. Weir and Churchil (1945) and Kapoor (1958) state that it is secreted by glands. In *Hilsha ilisha* also the presence of numerous glands in the pyloric region of the stomach may suggest that this protecting layer is probably secreted by glands.

The digestive tract of *Hilsa ilisha* is most interesting as for as its pyloric caeca are concerned, The number of caeca varies in different fishes and families. It varies from one (in Fistularia serrata, Amodytes) to 900 in *Merlangus carbonarius*. In *Lepidosteus* and *Acipenser* etc. the pyloric caeca are highly developed and complicated. The clustering of pyloric caeca is very characteristic in Clupeidae and above all in *Hilsa ilisha* the concentration seem to have reached its maximum limit as such a high concentration of pyloric caeca has not been recorded from any other fish.

Pyloric caeca are the outgrowths of the duodenum from where it arises as a process of evagination. Histologically the structure of pyloric caeca is similar to that of the intestine and so the author agrees with Rahimullah (1945) that the probable function which can be ascribed to them is that they act as a reservoir of semi-digested food material and also probably absorb the digested food matter like the intestine The structure of the pyloric caeca is simple throughout in *Hilsa ilisha* and is not complicated as shown in many other fishes by Rahimullah (1945). The ciliated row of very fine cilia which are accompanied with the mucosal epithelium towards its inner edge in *Heptacus nigricans* and *Pterois russelli* (as described by Rahimullah, 1945) are absent in *Hilsa ilisha*.

It may be mentioned here that the goblet cells are not known to occur in the stomach. Their concentration is maximum in the rectal region. Al-Hussaini (1945, '46 and '47) characterises such abundance of goblet cells as a distinguishing feature of rectum. Girgis (1952) and Purser (1928) have also observed that goblet cells are in plenty in the last portion of the gut.

SUMMARY

The alimentary canal of *Hilsa ilisha* consist of buccal cavity, short pharynx and oesophagus, cardiac and pyloric stomach, duodenum, intestine and rectum.

The buccal cavity is devoid of teeth as a result of which food materials are swallowed in as a whole.

The clustering of pyloric caeca is very characteristic in clupeidae and in *Hilsa ilisha* the concentration has reached its maximum. Such a high concentration has not been reported so far from any other fish.

There are two maximum feeding periods both in males and females alternating with a starvation or semi-starvation period. The maximum feeding period in case of males are March and September whereas in case of females it is March and August. Juveniles are voracious feeders.

Sexually matured Hilsa feeds during spawning migration.

Though the buccal cavity of *Hilsha ilisha* is devoid of taste buds yet it is not a blind-feeder. There is a rich supply of nerve fibres just below the mucosal epithelium which seem to be the primitive step in the development of the taste-buds.

Sense of taste is carried to the brain not only by the buccal cavity but also from the pharynx and oesophagus.

The upper jaw is devoid of such nerve supplies which show that it does not take part in gustatory sense.

Gill-rackers are characteristically modified and adapted for straining microscopic plankton from the water.

The cardiac stomach functions as a reservoir for food and the pyloric stomach functions as a true stomach.

There are highly developed gastric glands in the pyloric stomach. They are also present in the cardiac stomach but the number increases as we pass to the posterior region. The submucosa of the pyloric stomach is almost completely occupied by the gastric glands.

The goblet cells are completely absent from the cardiac and pyloric parts of the stomach and are scarce in the duodenum. Their concentration is maximum in the last portion of the gut (rectum).

ABBREVIATIONS USED

bl. v.	Blood vessel	b m.	Basement membrane
car.	Cartilage	car. s.	Cardiac Stomach
Cir. m.	Circular muscles	Col. c	Columnar cells
Col. epi.	Columnar epithelium	duo.	Duodenum
epi. c.	Epidermal cells	gas. g.	Gastric gland
gas. g. lu.	gastric gland lumen	gas. g n.	Neck of the gastric gland
g. b.	Gall bladder	gi.	Gills
gob. c.	Goblet cells	hrt.	Heart
int.	Intestine	l. liv.	Left liver
long. m.	Longitudinal muscles	muc.	Mucosa
mus. l.	Muscularis layer	n. f.	Nerve fibres
nuc.	Nucleus	Oes.	Oesophagus
P. aci.	Pancreatic acini	pn. d.	Pneumetic duct
P. L.	Protecting layer		
Pyl. cae.	Pyloric Caeca	Pyl. s.	Pyloric stomach
Pyl. cae. o	Pyloric Caeca opening	rec.	Rectum
r. liv.	Right liver	ser.	Serosa
Spl	Spleen	Sub-m.	Sub-mucosa
		st, epi.	Stratified epithelium.
tu pr.	Tunica propria	val.	Valves.

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*SEASONAL VARIATIONS IN THE OVARY OF *HILSA ILISHA* (HAMILTON) FOUND AT ALLAHABAD

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Variations in the size of gonads of vertebrates during different seasons of the year is of common occurrence and is a familiar fact. Teleosts also exhibit such changes in volume and weight of their gonads. A good deal of work has been done on cytological lines on problems of Oogenesis but the study of Cyclic changes in the morphology and histology of ovary of fishes has comparatively been able to attract much less attention. A survey of literature reveals that in India only some stray work has been done on the subject. The Indian shad *Hilsa ilisha* are found in Allahabad throughout the year and provide a good material for such a problem. The author has therefore made an attempt to collect some informations regarding the seasonal variations in the ovary of *Hilsa ilisha* to study the breeding habits of the fish.

Among the notable workers on the seasonal variations in the ovary of fishes are Cunningham (1897), Franz (1909), Wheeler (1924), Hann (1927), Mien (1927), Craig-Bennet (1931), Hickling (1935), Gryzeva (1936), Turner (1937), Mathews (1938), Bullough (1939), Suzuki (1939), Guerbilsky (1939), Mendoza (1939, 1940, 1941 and 1943), Robinson and Rugh (1943), James (1946), Chang (1949), Ghosh and Kar (1952), Dixit (1953) and Yamamoto (1956).

MATERIAL AND METHODS

Hilsa ilisha for the present work were collected from the local waters of Ganga and Jamuna. Collections were regularly made twice a week for more than two years. The data collected are based on the examination of 276 females. Fish were caught alive and weighed fresh on the spot, the total length and weight of each fish were noted. The fish were dissected and the ovaries of each fish were weighed to the nearest miligrams. Small slices of ovaries were fixed in Bouin's fluid and in Allen's modification of Bouin's fluid. After embedding in paraffin the blocks were cut at 6·8 mircons in thickness and stained with Heidenhan's iron alum haematoxylin counter-stained with eosin. Mellory's triple and Delafield's haematoxylin stains were also used but Heidenhan's iron alum haematoxylin proved more valuable.

The percentage of gonad weight/body weight relationship has been studied month wise and the results have been expressed in graph No. 1 based on Table I. This study has further been supplemented by the measurement of ova with a view to determine, if possible, the stage of maturation of the ova, diameter of 100 ova taken at random from each ovary were recorded. As no significant difference was observed the measuement of the different groups of ova from various parts of the ovary, ova were indiscriminately gathered from any part of the ovary without selection. The diameter of the eggs that fell in the line with the micrometer scale of the eye piece were measured and as such selection was eliminated.

* A part of the thesis approved for the degree of Doctor of Philosophy of the University of Allahabad in 1957.

James (1946), Mathews (1938), Ghosh and Kar (1952) and Dixit (1953), have recommended the percentage ratio of the gonad-weight to the body weight as a fairly constant and reliable criterian for studying the seasonal variations in the gonads. The author has therefore adopted a similar method.

Graph 1 evidently shows that the ratio of the gonad weight to the body-weight in females reaches its peak twice a year.

TABLE 1

Average weights of *Hilsa ilisha* (Females) taken in monthly collections from Ganga and Jamuna at Allahabad, with corresponding average weights of gonads, and calculated gonad-weight and body-weight ratio expressed as percentage of body weights.

Months		No. of specimens taken	Average weight of Fish	Average weight of ovary	Average ratio between ovary and body weight
			Gms.	Gms.	Percent.
January	...	17	689·53	6·44	0·93
February	...	20	648·6	29·29	4·5
March	...	34	670·97	38·92	5·8
April	...	20	740·18	11·76	1·59
May	...	28	478·90	3·43	0·72
June	...	17	743·17	30·84	4·15
July	...	10	782·79	39·76	5·08
August	...	11	765·30	55·24	7·22
September	...	15	842·25	70·78	8·4
October	...	33	884·80	108·37	12·25
November	...	40	808·66	81·76	10·1
December	...	31	715·42	16·23	1·43

The ovaries of Hilsa are suspended in the abdominal cavity by means of a thin mesentery. When fully developed the ovaries completely fill the body cavity. Matured ovaries rupture and the ova are liberated in the abdominal cavity from where they pass out through the urinogenital pore. If a piece of ovary is examined of this stage under the microscope, it will be seen that besides mature ova there also occur small immature ova which remain connected with the germinal epithelium.

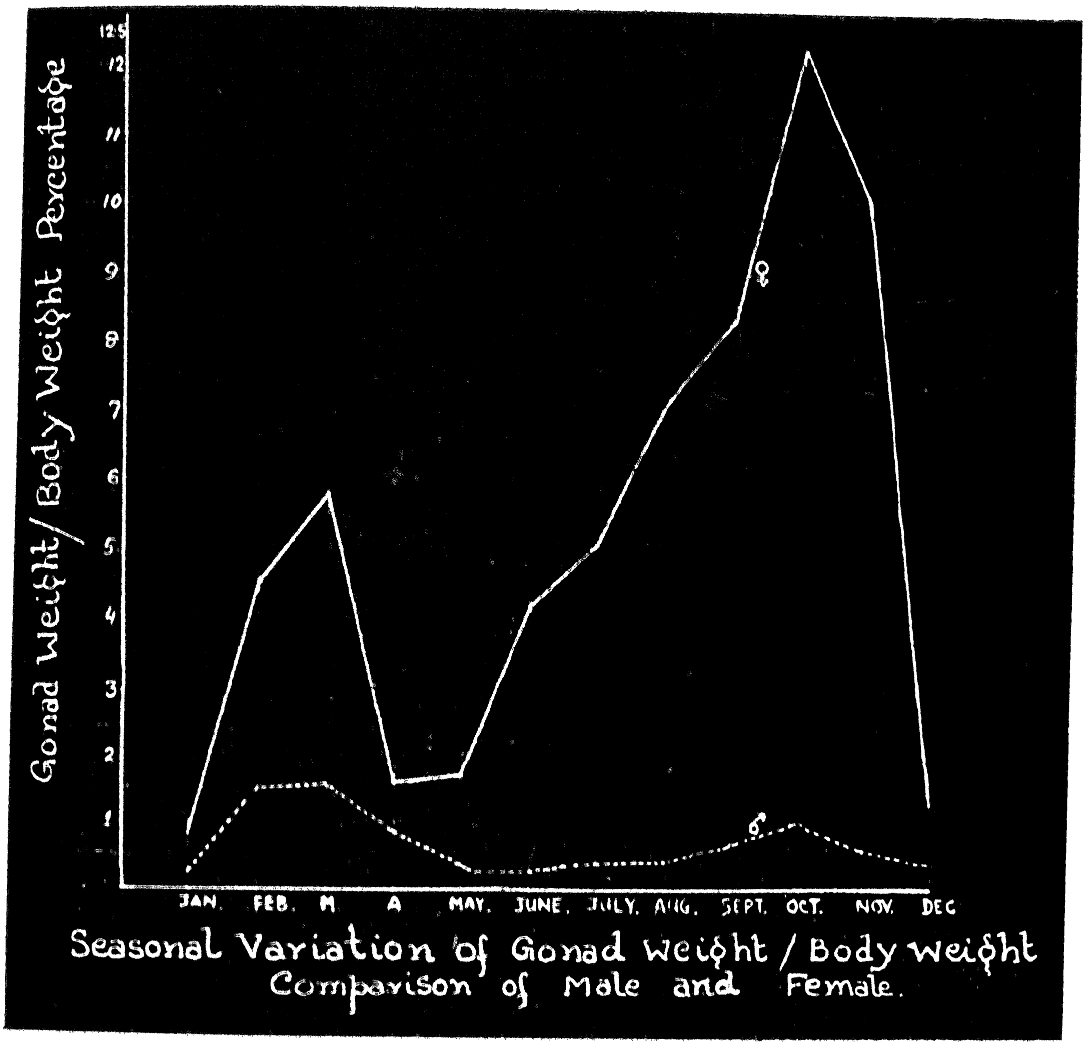

Fig. 1. Photomicrograph of the T. S. of immature ovary (Highpower).

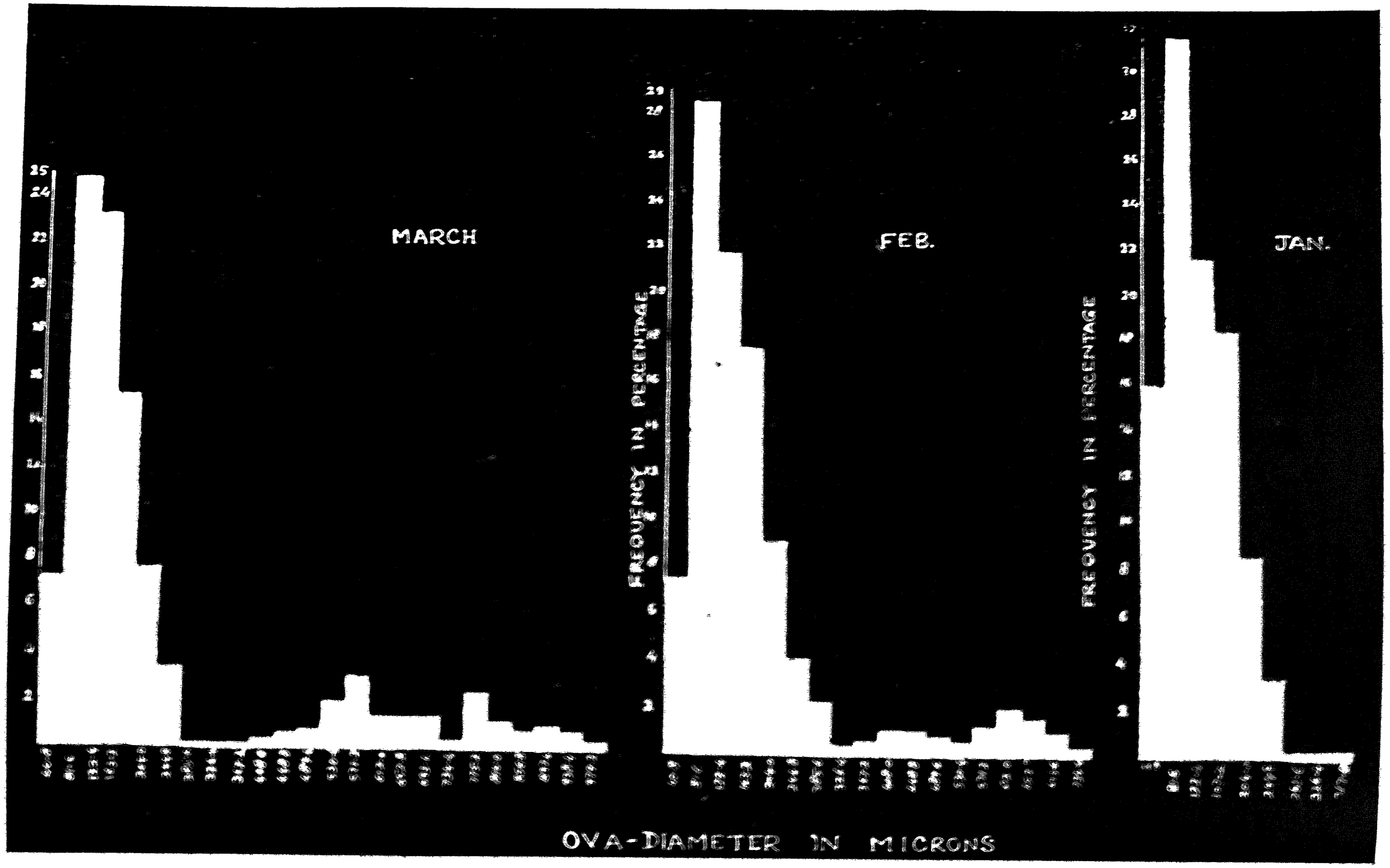

Fig. 2. Photomicrograph of the T. S. of a spent ovary (Low power).

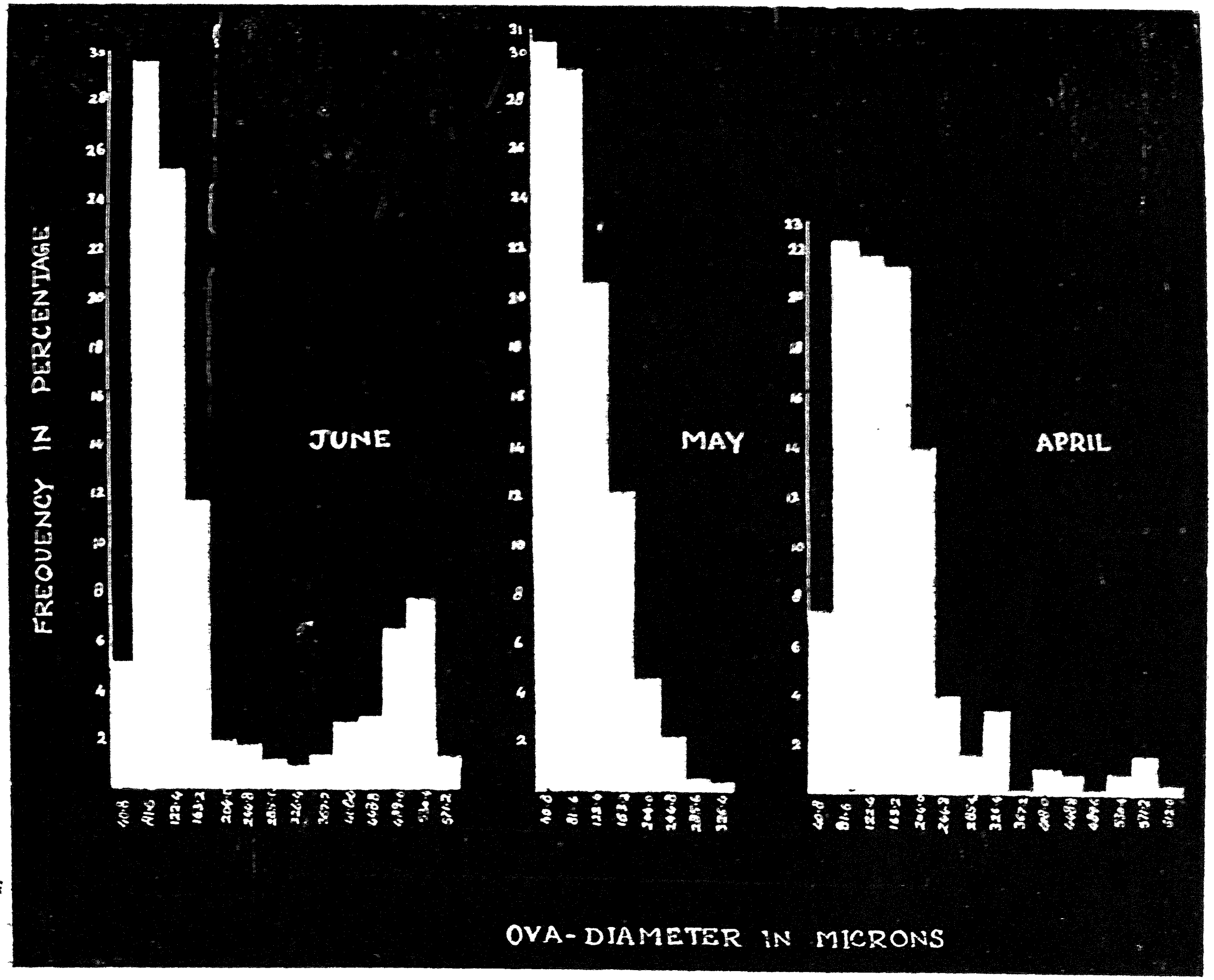

Fig. 3. Photomicrograph of the T. S. of ovary showing the arrangement of ova in ovigerous lamellae (low power). Beginning of vacuolization is seen.

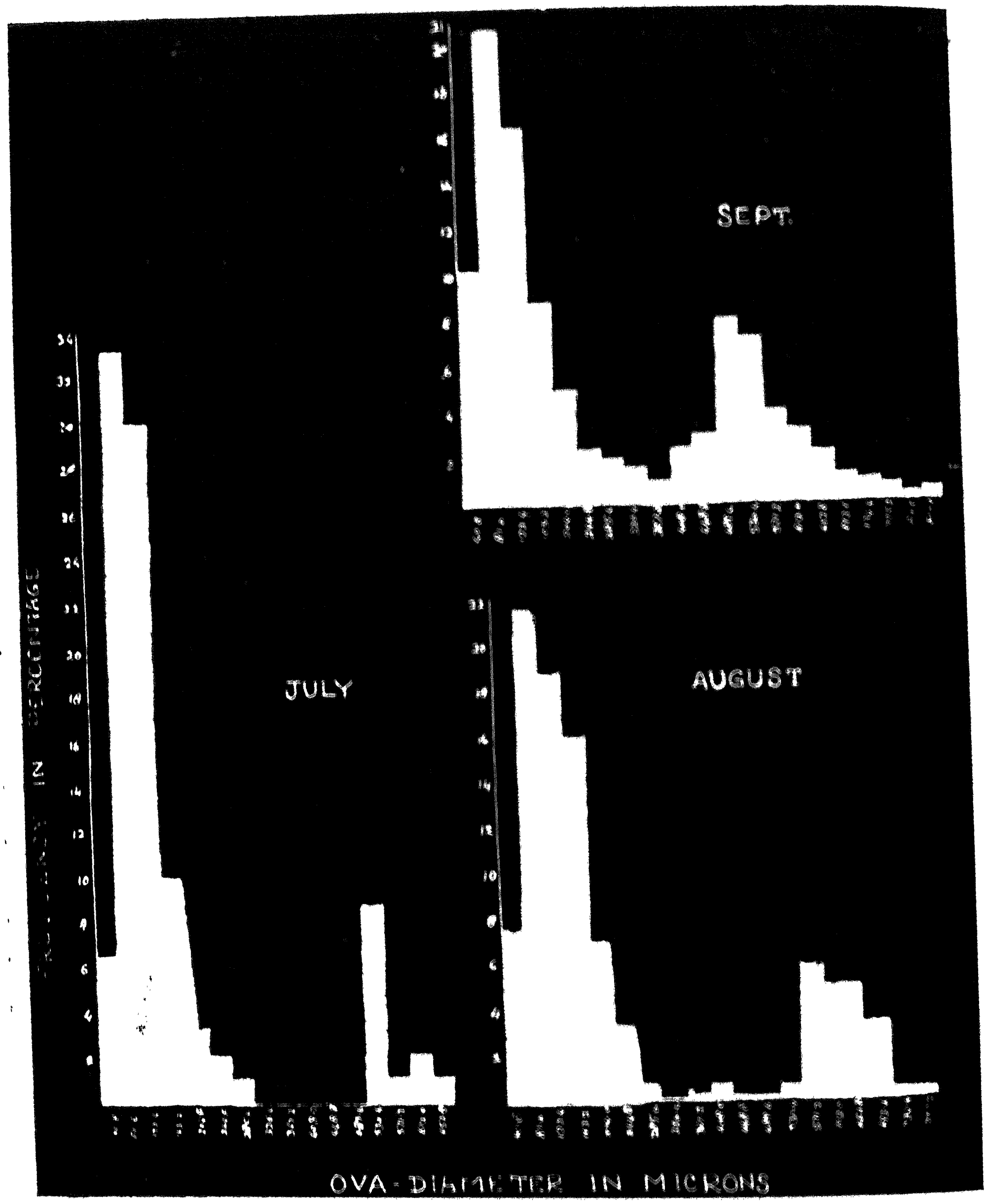

Fig. 4. Photomicrograph of the T. S. of ovary heading towards maturity showing yolk formation.

The ovaries have been given the following field classification :—

1st Stage :—Immature thread like ovaries indistinguishable from immature testes.

2nd Stage : —Ovaries extending upto half the length of the body cavity. They are maroon coloured, with indiscrete ova.

3rd Stage :—Ovaries occupying 2/3rd of the body cavity. The Minute ova can be seen with the unaided eye.

4th Stage :—Ovaries extending over almost the entire length of the body cavity and filling half of the body cavity. The ova are clearly seen with naked eyes.

5th Stage :—Ovaries filling the entire body cavity and the ova are of almond colour. The ova are pretty large in size.

6th Stage :—The only difference between the 5th and 6th stage is that in the latter the ova are seen oozing out of the vent even on slight pressure on the body cavity.

7th Stage :—Spent ovaries with immature ova.

HISTOLOGY

The oocytes are seen arranged in definite folds the ovigerous lamellae which project towards the centre of the ovary (fig. 3).

IMMATURE OVARY

They exhibit only the early stages of oogenesis. Primary and secondary oogonia are observed with large spherical nuclei. The cytoplasm around the nuclei are seen as thin pellicle. Lamp-brush chromosomes are distinctly seen in some of the oocytes. The ovigerous lamellae are not well defined (fig. 1).

For sake of convenience in studying the ovarian cycle, keeping in view the histological and morphological changes undergone by the ovaries the year has been divided into the following periods.

DECEMBER AND EARLY JANUARY

The ovaries are in spent condition with immature ova. The oocytes are seen arranged in ovigerous lamellae (fig. 2).

Some oocytes show indications of cytosomal differentiation and vacuoles are seen appearing in a circular band along the periphery (fig. 3). The cytoplasm of the oocytes of 1-68 microns in diameter takes deep blue haematoxylin stain. These oocytes have large nuclei with their nucleoli dispersed. The follicular epithelium is not distinct at this stage and can be seen just as a membrane under high magnification. In the oocytes measuring ·5 mm - 1·0mm., we sometimes observe yolk nucleus of Balbiani lying in juxta nuclear position. They are of various shapes—Crescentric, globular etc. and are seen before the vacuolization of cytoplasm sets in.

LATE JANUARY AND FEBRUARY

The oocytes are seen heading towards maturity falling within the range of 367 microns to 734 microns in diameter. The cytoplasm exhibit noticeable changes leading to total vacuolization (figs. 4, 5 and 6). The nucleoli are arranged along the nuclear walls of the oocytes. The oocytes display distinct follicular epithelium, closely applied to the vitelline membrane. Vacuolated oocytes are characterized by the presence of yolk globules in their cytoplasm.

As there is always a certain percentage of immature oocytes in the ovary of *Hilsa ilisha*, young oocytes measuring between 13 microns to 285 microns in diameter are also seen. A few atretic cells are also observed with their enlarged follicular cells invading the cytoplasm. Towards the end of February, oocytes increase in size and large spherical masses of yolk material are seen throughout the cytoplasm (figs. 5 and 6).

MARCH

The ovaries are fully matured (in 5th and 6th stages of maturity). The ova have attained a size upto 979 microns. The cytoplasmic area increases in bulk and the nuclei are proportionately very much reduced with their nucleoli arranged along the nuclear membrane. The ova are laden with yolk. The immature group of ova persist.

APRIL

We find ovaries in different stages of maturity depending upon the individual fish whether they have discharged or have partially done so or are about to lay eggs. The majority of the specimens examined have their ovaries in a condition corresponding to that prevelent in December and early January. In some of the early spawners a condition similar to that of late January is observed and in a few we find ovaries still with matured ova ready for spawning. Partially spent ovaries are also seen.

MAY & JUNE

Ovaries are in a similar condition as found in December and January.

JULY AND EARLY AUGUST

The ovaries resemble in their structure and stage of maturity to those of late January and February.

LATE AUGUST TO LATE OCTOBER

The ovaries are in a similar condition as in March.

NOVEMBER

Ovaries are exactly similar to those of April.

The study on seasonal variations in the ovary of *Hilsa ilisha* has been substantiated by the study of the seasonal progression in ova size. The variations in size of ova have been presented in a histogram pattern displaying the mean size frequencies computed for the various months (Vide Histograms).

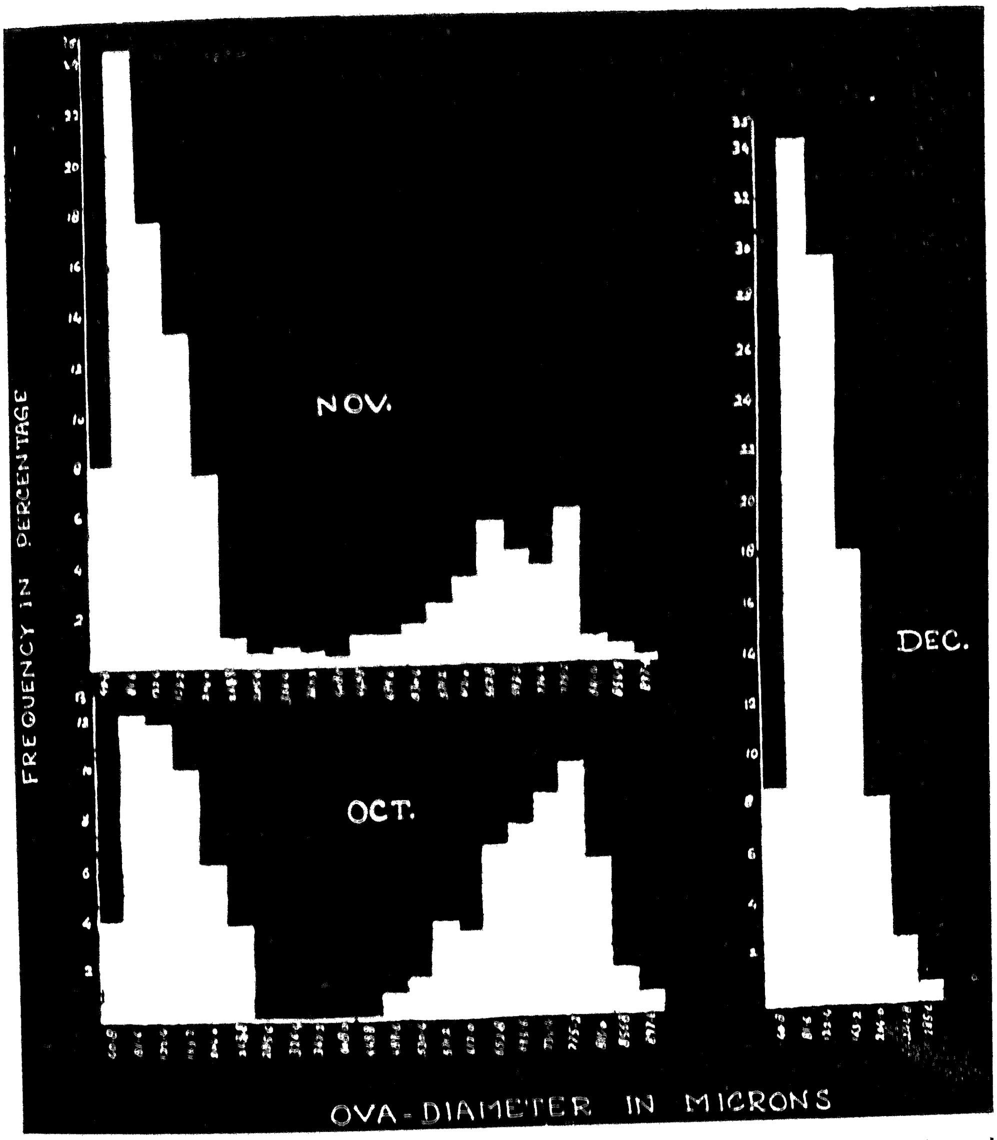

Fig. 5. Photomicrograph of the T. S. of ovary showing complete vacuolization of the cytoplasm with advanced stage of yolk formation (High power).

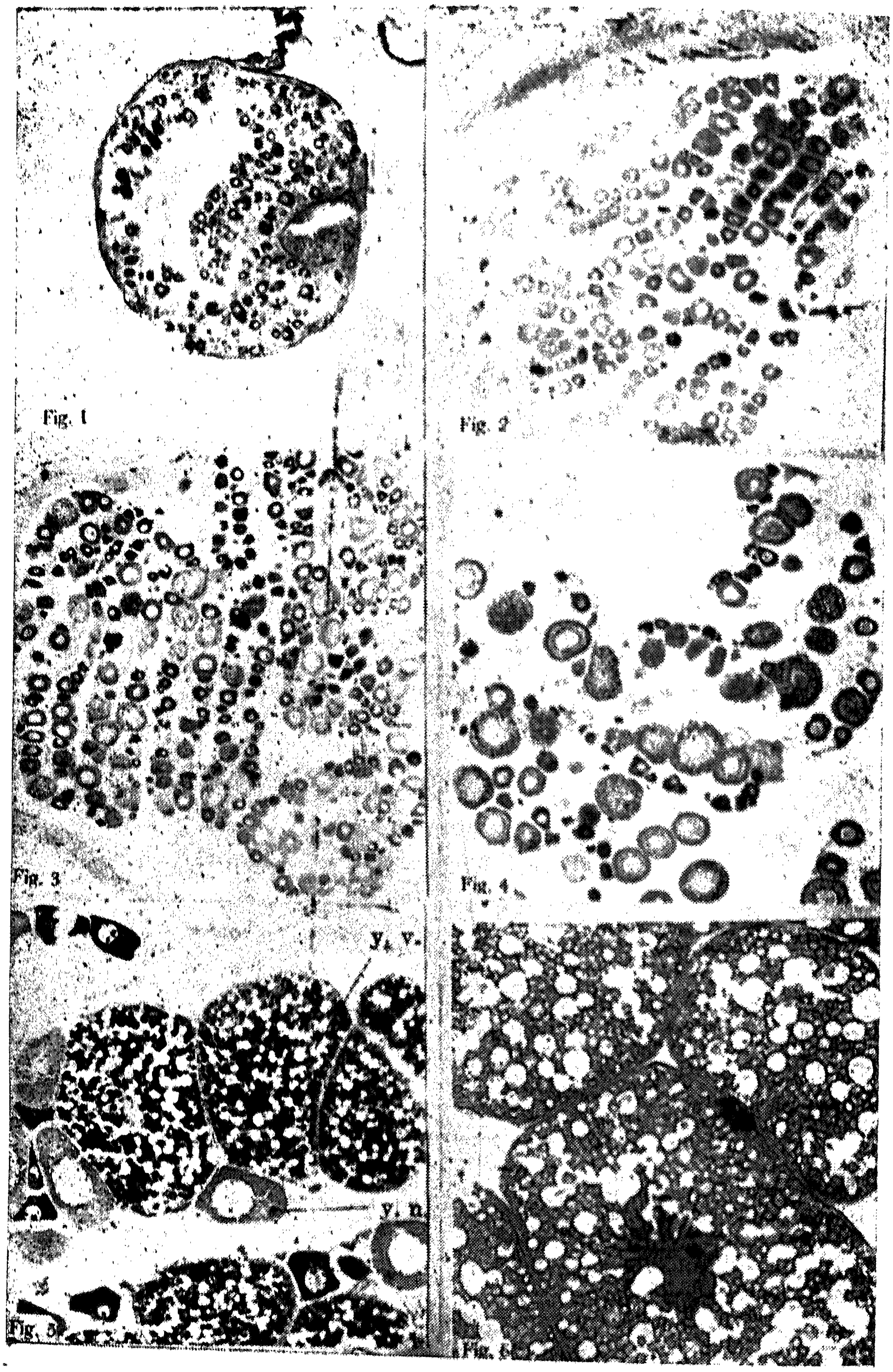

Fig. 6. Photomicrograph of the T. S. of mature ovary. Ova laden with yolk (High power).

The histograms show two groups of ova—(1) Immature ova upto 244·8 microns in size which are present as dominant group all round the year. The modal size of this group lies between 81·6 — 122·4 microns. (2) This group has a size range of 244·8 — 884 microns casually reaching upto 979 microns. They are obviously meant to be spawned during the ensuing spawning season.

From June onwards a steady increase in the maximum size of ova is observed but there is no marked increase in the modal size of ova from June to September which lies between 489·6 — 612 microns. In October there appears to be a graphical shifting in size and the modal size of ova increases to 775·2 — 816 microns. This is indicative of the fact that the first peak of shawning occurrs in the months of October and November.

The histograms of the succeeding months show that in December the spent specimens have only immature ova. The maturation cycle is repeated and from January onwards we find the ova increasing in size. By March the maturing group of ova reach the maximum size (upto 979 microns). In April and May the specimens caught again show spent ovaries. This shows that the second peak period for spawning occurrs in March.

DISCUSSION

It is quite evident from the graph — 1 that the ovaries like testes exhibit seasonal variations in relation to the weight of the fish. Allahabad *Hilsa* has two breeding seasons —(1) August to November ; (2) February to March. The ovary weights further indicate that the peak spawning months are October and March respectively. Histological studies are in conformity with the above observations.

In early stages of egg growth the nuclei are large in relation to the size of the eggs and lamp-brush chromosomes are noticed inside them. In the oocytes of about 68 microns in diameter the nuclei are oval and the nucleoli are seen dispersed. At this stage the cytoplasm takes a deep stain and is of basophilic nature.

As immature eggs grow in size their cytoplasm show signs of gradual vacuolization. These vacuoles are yolk vacuoles. The vacuoles make their first appearance along the periphery of the ova. Bullough (1939), James (1946) and Cooper (1952) have made similar observations in other fishes. The increasing degree of vacuolization of cytoplasm in the growing eggs is dependant on the stage of maturity of the ovaries and the increase in size and weight of ovaries is due to the deposition of yolk material. Figs (3—6) show different stages of Vacuolization.

Fully mutured ova laden with yolk are met within the ovary of *Hilsa ilisha* twice a year——*viz.*, from August to November and from February to March, (during the two breeding seasons). The peak of the ovarian activity is seen in October and March respectively. The cycle of maturation is repeated after every spawning season. This observation is further supported by the study of the seasonal progression of egg growth.

The wide range of size (244·8 microns to 938 microns) covered by the maturing group of ova meant to be spawned during the spawning season instead of a distinct group of matured ova, and the frequent occurrence of partially spent ovaries during the spawning seasan bear testimony to the fact that the females of *Hilsa ilisha* do not have a single spawning act. They seem to release their ova in instalments for several times during a spawning season.

Lehman (1953) describes a similar behaviour in the Hudson river shad and states "From the variations in size of the maturing ova it appeared that the shad have a multi-spawing or a continuous spawning rather than a single spawning act'. According to Rosa (1957) Pillay in 1956 also arrived at a similar conclusion while working on Hoogly river Hilsa.

In ovaries at early stages of maturity certain oocytes exhibit the presence of yolk nucleus of Balbiani. The yolk nucleus has more affinity for stain than the surrounding cytoplasm. As the present study does not involve any cytological study the author does not propose to discuss about its functions. Moreover their functional significance seem to be obscure as there already exist much controversy on the subject.

Coming to the atretic eggs, we find that mostly they have been reported from the viviparous fishes (Turner 1937, Mathews 1938 and Mendoza 1943). So far as the modes of atresia are concerned the author finds himself in agreement with Turner (1937) and Dixit (1953). The follicle cells become somewhat stellite in appearance and invade the enclosed egg cytoplasm from sides and finally the space formerly occupied by the oocytes become a mass of debris filled up with rounded cells which were follicle cells. The atretic eggs are much less in *Hilsa ilisha* as compared to viviparous fishes reported by Turner (1937) and Mendoza (1943). This may be considered as an adaptation by the oviparous fishes as the chances of wastage of eggs is much more in them due to the fertilization being external.

The interstitial cells are altogether absent in the ovaries of *Hilsa ilisha*.

SUMMARY

1. There is conspicuous seasonal variation in size and development of the ovary of *Hilsa ilisha*.

2. Allahabad Hilsa has two breading seasons—

   (*a*) August to November and (*b*) February to March with the peak spawning periods in October and March respectively.

3. There is a gradual increase in the Vacuolization of the Cytoplasm of the growing eggs. The increase in Vacnolization is directly dependant on the stage of maturity.

4. Before spawning the Ovaries are full of yolk material resulting in their marked enlargement.

5. Hilsa spawns several times during a spawning season and do not have a single spawing act.

6. Interstitial cells are altogether wanting in the ovary of *Hilsa ilisha*.

ACKNOWLEDGEMENTS

I am grateful to the late Professor D. R. Bhattacharya formerly Vice-Chancellor of Allahabad University for suggesting this problem and to Dr. S. K. Dutta for his guidance. I am also thankful to the Council of Scientific and Industrial Research, India for financial assistance.

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STUDIES ON SOME CESTODE PARASITES

III. VARIABILITY IN THE NUMBER AND POSITION OF TESTES IN SOME UNARMED SPECIES OF *HYMENOLEPIS* FROM MAMMALS

By

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[Received on 19th February 1959]

During a course of investigation on a number of unarmed species of the genus *Hymenolepis* Weinland, 1858, I found that the number of testes and their position, more so the latter, are extremely variable. In the past little or no importance was given to this aspect of morphology till Mayhew (1925) proposed his three genera. Occasional references of testicular variation have, no doubt, been brought to light by Sturdevant (1907), Fuhrmann (1924 and 1932), Meggitt (1927), Meggitt and Subramanian (1927), Schiller (1950) and Singh (1956); most of them having made no attempt to study the incidence of such abnormalities. Nevertheless, Palais (1933) has, in her extensive work, reported a number of variations in *Hymenolepis diminuta* Rudolphi, 1819 in France. In the United States, Voge (1952) undertook a thorough study of various variants in specimens of *H. diminuta*, *H. citelli* McLeod, 1933 and *H. horrida* von Linstow, 1901 in order to determine the relationships between these species. It has been noted that *H. diminuta* had been one of the commonest material to be investigated upon. No such study has ever been attempted in this part of the world and, therefore, I decided to study the incidence of abnormalities occurring in *H. diminuta* from rats, *H. minimedius* n. sp. from bats and *H. palmarum* Johri, 1956 from the palm squirrel.

METERIALS AND METHODS

Specimens of *H. diminuta* were obtained from two different host species. Of the two host species of *Meltada millardia* Gray collected at Hardoi, only one yeilded six specimens, many without gravid segments. Two host species of *Mus buduga* Gray caught in Lucknow yeilded another six specimens of parasites out of which two were incomplete ones. *H. minimedius* n. sp. was collected from the intestine of a vampire bat, *Pteropus medius* Temminck shot at Bareilly U. P. Three specimens of *H. palmarum* Johri, 1956 previously obtained from the palm squirrel, *Funambulus palmarum* Linn. were the only ones available for study. The worms were fixed in 5% formaline in R. L., corrosive sublimate solution and Bouin's fluid and were thoroughly washed in a stream of running water usually overnight and were finally preserved in 70% alcohol. Ehrlich's haematoxylin, Acetic acid alum carmine, Borax carmine and Semicon's carmine were used as stains for whole mounts. Material for sections after routine dehydration and embedding in paraffin was cut 5-8 μ in thickness. The sections were stained either with Ehrlich's haematoxylin or Heidenhann's iron haematoxylin and Eosin.

Hymenolepis diminuta Rudolphi, 1819

(*a*) Specimens collected at Hardoi from *Meltada millardia*

(*b*) Specimens collected at Lucknow from *Mus buduga*

The normal pattern of testes found in this species is triangular ; one poral and two aporal, the latter ones situated one behind the other though the distance between them may vary in different segments. The following types of testicular variations in their number and position have been observed in this species.

(Symbols 'p' and 'ap' denote the positions of the poral and the aporal testes)

First type : Number—1 poral (1 p) and 2 aporal (2 ap).

The variation takes place in the position of the aporal testes which deviate from the normal triangular condition and acquire more or less a diagonal position. The posterior aporal testis shifts towards the ovary lying almost above it and nearly central or median in position. The percentage of shift in materials (*a*) and (*b*) is 12·42 and 13·12 respectively. (Tables I and II)

Second type : Number—1 poral (1 p) and 1 aporal (1 ap).

The chief variation is in the number of testes which are reduced to two (*Diorchis* type). This change is brought about by the disappearance of the anterior aporal testis of the normal pattern and the two remaining testes are almost in a straight line. The percentage of reduction in the number of testes in materials (*a*) and (*b*) is 6·05 and 5·36 respectively.

Third type : Number—1 poral (1 p) and zero aporal (0 ap).

In this variation, a single testis is present on the poral side touching the posterior border of the segment (*Aploparaksis* type). The aporal testes are missing. This variation occurs only in material (*a*) from Hardoi, the material (*b*) from Lucknow is normal, the percentage of the variant being 0·31 only.

Fourth type : Number—1 poral (1 p) and 3 aporal (3 ap).

In this variation there is an addition of one testis to the normal number bringing the total to four testes (*Oligorchis* type). The percentage of this variation in materials (*a*) and (*b*) is 0·77 and 1·89 respectively.

Fifth type : Number—Zero poral (0 p) and 1 aporal (1 ap).

In this variation a single testis is present touching the posterior border of the segment on the aporal segment (*Aploparaksis* type). Evidently it is the case where two testes, one poral and one aporal have disappeared. The percentage of variation in materials (*a*) and (*b*) is 0·15 and 0·55 respectively.

Sixth type : Number—Zero poral (0 p) and 2 aporal (2 ap).

In this variation the poral testis from the normal pattern is missing and the total number of testes is reduced to two (*Diorchis* type). The two testes lie one behind the other as in a normal condition. The percentage of variation in materials (*a*) and (*b*) is 3·26 and 0·28 respectively.

Seventh type : Number—Zero poral (0 p) and 3 aporal (3 ap).

In this variation there are three testes, all aporal in position, there being not a single testis on the poral side. The three testes present show a triangular pattern. The percentage of variation in materials (*a*) and (*b*) is 3·10 and 0·28 respectively.

Eighth type : Number—2 poral (2 p) and 1 aporal (1 ap).

In this variation though the number of testes is normal, the position of the three testes show the reverse condition from the normal pattern. The two poral testes usually lie one behind the other. The percentage of variation in materials (*a*) and (*b*) is 0·77 and 0·04 respectively.

Ninth type : Number—2 poral (2 p) and 2 aporal (2 ap).

In this variation there is an addition of a poral testis bringing the total to four (*Oligorchis* type). The percentage of variation in materials (*a*) and (*b*) is 0·62 and 0·04 respectively.

Hymenolepis minimedius n. sp.

The normal pattern of the testes here is a straight line arrangement (transverse); one of these is poral and the other two are aporal. All the three testes are situated close to each other and almost touching the posterial border of the segment within the ventral longitudinal excretory vessels. The following types of variation have been observed in this species. (Table III and VI)

First type : Number—1 poral (1 p) and 2 aporal (2 ap).

This variation is distinguishable into two types :—

1. This pattern shows a variation from the normal type in the shifting of the aporal (central) testis towards the ovary thus slightly disturbing the transverse arrangement of the testes. The percentage of shift is 2·46.

2. This pattern shows the two aporal testis arranged one behind the other, roughly representing a triangular arrangement of all the three testes. The percentage of variation is 1·11.

Second type : Number—1 poral (1 p) and 1 aporal (1 ap).

In this variation the number of testes is reduced to two (*Diorchis* type). The aporal (central) testis of the normal pattern disappears and the two remaining testes are situated normally in a straight line separated by the female genital organs. The percentage of reduction is 1·48.

Third type : Number—1 poral (1 p) and 3 aporal (3 ap).

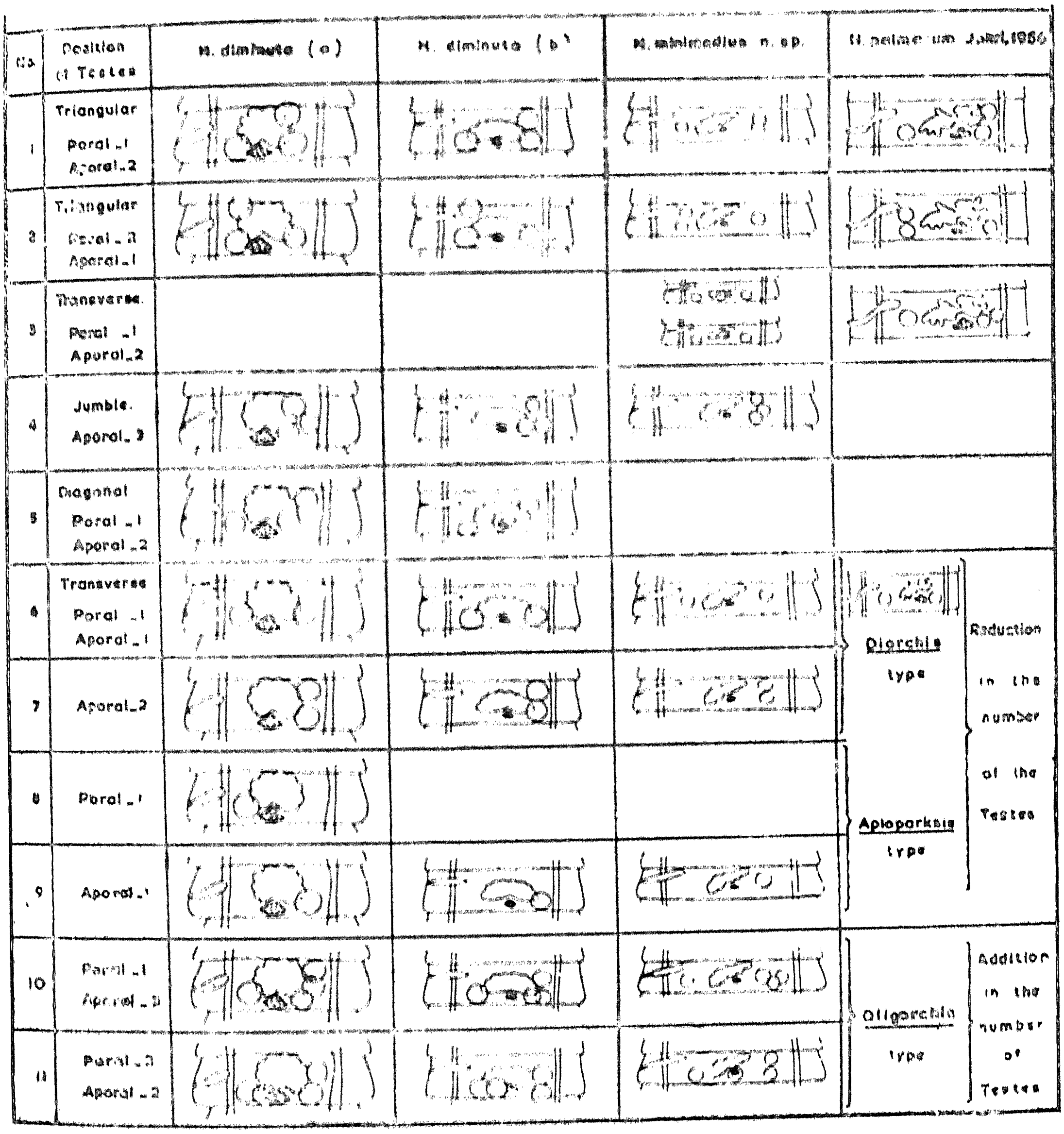
In this variation there is an addition of one aporal testis bringing the total to four (*Oligorchis* type). There is a slight shifting of the central (aporal) testis and all the three aporal testes are in a jumble situated between the ovary and the ventral aporal longitudinal excretory vessel. The percentage of variation is 0·18.

Fourth type : Number—Zero poral (0 p) and 1 aporal (1 ap).

In this variation a single testis is only left on the aporal side (*Aploparaksis* type). It normally lies between the ovary and the ventral aporal longitudinal excretory vessel. The percentage of variation is 0·27.

Fifth type : Number—Zero poral (0 p) and 2 aporal (2 ap).

VARIATION IN THE POSITION & NUMBER OF TESTES IN HYMENOLEPIS



In this variation two aporal testes are present located one behind the other (*Diorchis* type). The poral testis of the normal pattern is absent and the central (aporal) testis shifts towards the second aporal testis. The percentage of variation is 1·57.

Sixth type: Number—Zero poral (0 p) and 3 aporal (3 ap).

In this variation there are three testes, all aporal in position in a jumble between the female genital organs and the ventral aporal longitudinal excretory vessel. There is no testis on the poral side. The central (aporal) testis also shows a shift from its normal position. The percentage of variation is 0·83.

Seventh type: Number—2 poral (2 p) and 1 aporal (1 ap).

In this variation though the member of testes is normal, their position shows a reverse condition of the first variant. In most cases the central (aporal) testis is displaced towards the poral side thus pushing the original poral testis towards the anterior part of the segment. The percentage of variation is 1·48.

Eighth type: Number—2 poral (2 p) and 2 aporal (2 ap)

In this variation there is an addition of an aporal testis near the aporal ventral longitudinal excretory vessel usually anterior to the normal second aporal testis bringing the total to four (*Oligorchis* type). The percentage of variation is 1·48.

Hymenolepis palmarum Johri, 1956

This species shows the least variation in comparison to the other two species already described. The normal pattern here is a straight line arrangement (transverse) of all the three testes of which one is poral and the other two aporal, always within the ventral longitudinal excretory vessels. The following types of variation have been observed in this species. (Table IV and VII).

First type: Number—1 poral (1 p) and 2 aporal (2 ap).

In this variation the two aporal testes are situated one behind the other representing a triangular arrangement of all the three testes thus deviating from the normal straight line arrangement. The percentage of variation is 0·81.

Second type: Number—1 poral (1 p) and 1 aporal (1 ap).

In this variation the number of testes is reduced to two (*Diorchis* type). One of the aporal testis has disappeared. The two testes are usually in a straight line. The percentage of variation is 0·97.

Third type: Number—2 poral (2 p) and 1 aporal (1 ap).

In this variation a condition opposite of variant no. 1 is obtained. There is a reversal in the number of the poral and the aporal testes. The two poral testes are usually situated one behind the other. The percentage of variation is 1·54.

DISCUSSION

The genus *Hymenolepis* contains over 400 species from both mammals and birds. While the species described from birds are armed, the mammalian forms are both armed and unarmed. The absence of an armed rostellum (and also its absence in a number of forms) removes away a very definite and reliable character for the routine identification of the species. Since the genus has become too unwieldy, some of the workers have, of late, started identifying the species by comparing with forms from a particular order or family alone of the host. Although such a criterion may be convenient, it is nevertheless, questionable.

As a result of the present study, it has been ascertained that, with regard to the arrangement of the testes, it is certainly true that in some species at least their position is quite stable although it is not the case in other species. The position of the testes in *H. minimedius* and *H. palmarum* shows a rather remarkable degree of stability though the study is based upon a limited number of strobilae only. The testicular variations can, therefore, be easily termed as fluctuations; while those occurring in both the samples of *H. diminuta* show relatively higher percentage of variation and are, therefore, worthy of attention. By far the testicular variations in *H. diminuta* are the highest. Testicular variations in *H. diminuta* have also been well pronounced in investigations made by Palais (1939) and Voge (1952). Comparing the percentages of various testicular variants in *H. diminuta* obtained in the present study with those reported by Voge (1952), it appears that excepting for the 0p 3ap condition which is significantly different, the other variants almost show a parallel percentage within certain limits.

Again it is questionable whether the study of testicular variation can aid in the identification of the species. Though it is somewhat certain that if the variability is normal, it becomes part of the character of the species, however, unless a large number of specimens from different hosts are examined, no definite conclusions could be drawn. Rausch and Tiner (1948) considered *H. citelli* McLeod, 1933 as a synonym of *H. diminuta* due to the occurence of a high degree of variability in their characters and the absence of sound differential points. Voge (1952) disagrees with this view and observ d prominent differences in the relative frequency of testicular and other morphological variants. There may be instances where two species may be morphologically alike in the adult state while their life cycles and larval stages may be quite different. It is my opinion, that in many instances, the elucidation of life cycles may be the only way to determine specific identity in such forms but they can be of value only when distinct differences can be observed.

What are the conditions bringing about such variations? It is highly probable that conditions favouring rapid wrowth of strobila may be to some extent responsible for the increase in variability, or it may be due to environmental influence though such studies are not much known. Goodchild (1958) made some experiments on the transfaunation of *H. diminuta* and reports the occurrance of normal strobilae in the worms recovered.

Though in my opinion it is not truly justifiable to accept differences in relative frequency of a single variant as the sole criterion for specific purposes, it is advisable that the variations be studied fully and differences worked out as in the case of other morphological characters normally used for the identification of species.

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TABLE I

Variation in number and position of testes in *Hymenolepis diminuta (a)*

No.	Length of the specimen	Total no. of segments	1p* 2a	1p** 2a	2p 1a	3a	1p 1a	1a	1p	1p 3a	2a	2p 2a	Variation in one specimen
1	44 mm.	75	50	8	0	3	11	0	0	0	3	0	25
2	42 mm.	81	58	15	2	1	2	0	0	2	1	0	23
3	63 mm.	86	63	13	2	0	5	1	0	0	1	1	23
4	38 mm.	74	47	9	0	2	2	1	0	2	9	2	27
5	48 mm.	140	111	19	1	1	5	0	0	0	3	0	29
6	74 mm.	188	138	16	0	13	14	0	1	1	4	1	50
Total		644	467	80	5	20	39	2	1	5	21	4	177

* Triangular arrangement. Normal pattern.

** Diagonal arrangement of all the three testes.

TABLE II

Variation in number and position of testes in *Hymenolepis diminuta (b)*

No.	Length of the specimen	Total no. of segments	1p* 2a	1p** 2a	2p 1a	3a	1p 1a	1a	1p 3a	2a	2p 2a	Variation in one specimen
1	142 mm.	160	123	17	0	1	12	3	0	4	0	37
2	59 mm. (incom.)	40	30	5	0	2	1	0	0	2	0	10
3	93 mm. (incom.)	115	91	11	0	1	9	0	0	1	2	24
4	146 mm.	188	149	20	0	4	9	0	4	2	0	39
5	127 mm	224	180	25	0	5	5	0	8	1	0	44
6	116 mm.	168	128	16	2	0	12	2	5	3	0	40
	Total ...	895	701	94	2	13	48	5	17	13	2	194

* Triangu ar arrangement. Normal pattern.

** Diagonal arrangement of all the three testes.

TABLE III

Variation in number and position of testes in *Hymenolepis mixirnedius* n. sp.

No.	Length of the specimen	Total no. of segments	*1p 2a	1p** 2a	1p*** 2a	2p 1a	3a	1p 1a	1a	1p 3a	2a	2p 2a	Variation in one specimen
1	20 mm.	98	78	2	4	5	1	4	1	1	2	0	20
2	19 mm.	108	97	1	2	2	2	1	0	0	3	0	11
3	16 mm.	81	72	0	2	0	0	2	0	0	5	0	9
4	20 mm.	102	93	2	3	0	1	1	0	0	1	1	9
5	14·5 mm.	90	83	1	2	1	0	1	0	0	2	0	7
6	18·2 mm.	106	96	0	5	4	0	1	0	0	0	0	10
7	17·8 mm.	95	83	0	1	2	1	0	0	0	2	1	7
8	19 mm.	105	92	3	3	0	3	2	1	0	1	0	13
9	15·1 mm.	85	79	1	2	0	1	0	0	1	0	1	6
10	17·4 mm.	92	86	1	1	1	0	1	0	0	0	2	6
11	12 mm. (incom.)	57	54	1	0	0	0	0	1	0	1	0	3
12	13 mm. (incom.)	60	55	0	1	1	0	3	0	0	0	0	5
	Total ...	1079	950	12	26	16	9	16	3	2	17	5	106

* Transverse arrangement. Normal pattern.

** Triangular arrangement.

*** The central (aporal) testis slightly anterior.

TABLE IV

Variation in number and position of testes in *Hymenolepis palmarum* Johri, 1956.

No	Length of the specimen	Total no. of segments	1p* 2a	1p** 2a	2p 1a	1p 1a	Variation in one specimen
1.	256 mm.	259	252	2	3	2	7
2.	247 mm.	234	225	2	5	2	9
3.	59 mm.	122	117	1	2	2	5
	Total	615	594	5	10	6	21

* Straight line arrangement. Normal pattern.

** Triangular arrangement of all the three testes.

TABLE V

Percentage of variants in *Hymenolepis diminuta* (*a*) and (*b*)

No.	poral testes	aporal testes	No. of segments with variants in (a)	No. of segments with variants in (b)	Variation percentage in (a)	Variation percentage in (b)
1.*	1	2	80	94	12·42	13·12
2.	2	1	5	2	0·77	0·04
3.	0	3	20	13	3·10	0·28
4.	0	2	21	13	3·26	0·28
5.	0	1	2	5	0·31	0·55
6.	1	1	39	48	6·05	5·36
7.	1	3	5	17	0·77	1·89
8.	1	0	1	—	0·15	—
9.	2	2	4	2	0·62	0·04
			177	194	27·45	21·56

* Diagonal arragement of all the three testes.

TABLE VI

Percentage of variants in *Hymenolepis minimedius* n. sp.

No.	poral testes	aporal testes	No. of segments with variants	Variation percentage
1.*	1	2	12	1·11
2.**	1	2	26	2·40
3.	2	1	16	1·48
4.	0	3	9	0·83
5.	0	2	17	1·57
6.	0	1	3	0·27
7.	1	1	16	1·48
8.	1	3	2	0·18
9.	2	2	5	0·46
			106	9·78

* Triangular arrangement of all the three testes.

** The central (aporal) testis slightly anterior.

TABLE VII

Percentage of variants in *Hymenolepis palmarum* Johri, 1956.

No.	poral testes	aporal testes	No. of segments with variants	Variation percentage
1.*	1	2	5	0·81
2.	2	1	10	1·54
3.	1	1	6	0·97
			21	3·42

* Triangular arrangement of all the three testes.

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